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SEROLOGICAL EVALUATION OF IMMUNE RESPONSES TO REPOSITORY INJECTION OF RAGWEED EMULSION

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THERE HAS BEEN a great interest in the repository or single injection method of treatment in allergy following recent reports of successful results by Brown,[1-6] Loveless,[7,8] and others.[9,10] However, despite the large number of patients included in these various studies, there has been no objective method for evaluating results of such repository injections other than the usual clinical impression of both the patient and physician, plus the usual recording of symptoms and medication during the pollen season. Brown has pointed out that in his studies, all patients exhibiting the slightest symptoms during the season have been classified as failures.[11] This has not been the method used for evaluation by others.

There have been an increasing number of reports suggesting the feasibility of using a passive hemagglutination procedure as a method for measuring anti-ragweed responses following therapy. Antibodies which will agglutinate ragweed-coated-erythrocytes apparently appear in high titers in treated subjects as opposed to non-treated subjects.

In vitro methods for measuring immune responses in allergy have been the goal of many investigations. Since the early reports of Coca and Grove[25] that precipitins are not found in sera of treated or untreated allergy subjects, there has been a great deal of work on attempts to devise and standardize other reproducible serological procedures. Rabbits have been found to form high levels of anti-ragweed precipitins. Attempts have been made to "co-precipitate" human anti-ragweed antibodies with such rabbit

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serum and ragweed extracts. However, both increases or decreases of the amount of such precipitates formed by rabbit anti-ragweed sera and ragweed pollen extract pre-incubated with allergic human sera have been reported.[13,14] Similar variable results have been obtained with complement fixation and complement-fixation-inhibition procedures.[15-17] Coupling of colloidal particles coated with ragweed extract has been observed,[18] but attempts to confirm this have failed.[19]

Red cells as passive vehicles for agglutination reactions have been utilized with increasing enthusiasm. A modified Coombs test had been utilized in earlier trials.[20] Recently, several groups have used the Boyden technique[21] of absorbing pollen extracts to tannic acid treated red cells.[22,23] Conjugation of ragweed extracts to similar red cells by means of bis-diazotized benzidine (B.D.B.), first used by Pressman *et al*,[24] then by Stavitsky and Arquilla,[25] and adapted to the pollen system by Sehon,[26-29] has found wide application with various allergens.

Recent reports by Arbesman,[30-32] who has used both the tannic acid and the B.D.B. method with the same serum samples, have indicated a major discrepancy in results between the methods. No correlation could be found between such titers and clinical responses, or even between titers themselves, in a series of patients treated by conventional aqueous extract methods. However, it did appear, according to both Sehon's group with the B.D.B. method, and according to Arbesman's results with the tannic acid method, that there is a general and a consistent increase in hemagglutination titers in sera of treated patients following aqueous therapy.

Despite this reported lack of correlation between methods, and the confusion as to the nature or importance of such hemagglutination antibody titers, it was thought of interest to follow closely such hemagglutination titers in a small group of hay fever patients being given repository ragweed emulsion treatment. Previous work with over four hundred patients treated with repository injections had indicated that, as compared to a group of over three hundred aqueous extract treated patients,[33] there was consistently a comparable or increased titer as measured by the B.D.B. method in such patients.

This report is based on a representative group of respository treated patients for whom adequate amounts of serum samples were available. The patients were those included in a nationwide cooperative study with aims to determine efficacy, safety, and other factors concerned with repository therapy. Hemagglutination titers of sera obtained prior to and following repository injection of such patients were determined by both the tannic acid and the B.D.B. methods using both giant and short ragweed extract to coat rabbit red blood cells. Control patients given aqueous injections and other groups of persons given repository injections for whom sufficient serum samples were not available for these extensive serological studies are not included in this report.

METHODS AND MATERIALS

Ragweed Extract and Emulsion.—A single source of pollen extract, emulsifying material, testing solutions, needles, syringes, and techniques for testing and administering were standardized and available from the American Academy of Allergy. The extract was a mixture of giant and short ragweed, 20 per cent w/v, extracted with Coca's solution, plus merthiolate added as a preservative. The concentrated extract contained 200,000 pollen units (P.U.) or 77,300 protein nitrogen units (P.N.U.) per ml. For skin and eye tests, a series of three-fold dilutions, starting with a 1:20 (50,000 P.U. or 19,350 P.N.U.) and progressing to a 1:393,660 dilution (3 P.U. or 1 P.N.U.) were available. The emulsion contained one part aqueous extract with two parts Arlacel-Drakeol mixture. This was obtained mixed, but not emulsified, in sterile volumes in various strengths so that a constant volume of 1 ml could be prepared for any desired dosage. Emulsions were prepared a day prior to injection by the use of a double-hubbed 18 gauge needle. At least one hundred exchanges were made by hand. Emulsions were checked prior to use as to the following: (1) gross appearance; (2) microscopic appearance (uniform emulsion with particle sized between 0.1 and 0.25 microns); (3) drop-in-water test (failure to spread on surface of cold water): and (4) scratch test on known ragweed sensitive cases yielding negative results.

Injections.—Injections were given six weeks prior to the start of the 1960 pollinosis season. All treatments were given in the form of a 1 ml subcutaneous injection in the deltoid area. A sterile, disposable syringe and a disposable twenty-three gauge needle were used. All patients remained under observation for one hour after injection and were given antihistaminic and anti-asthmatic drugs to be taken in the event of untoward reactions. A series of scratch and ophthalmic tests was obtained prior to the injection, just before the ragweed season, and after the end of the season.

Subjects.—All subjects were classified as being afflicted with ragweed hayfever according to the following criteria: (a) a positive history; (b) a positive scratch test and (c) a positive ophthalmic test. These patients were divided into three groups:

Group 1—Those who had been on perennial treatment—eighteen cases.

Group 2—Those who had received previous pre-seasonal therapy—fifteen cases.

Group 3—Those with no previous treatment—six cases.

TREATMENT

Perennial and Previously Treated.—A multiple of the dose which the previously treated patient could tolerate was given. In the usual procedure, an emulsion dosage containing 50-100 times the prior maximum aqueous

dose was used. Thus, if a patient could tolerate 200 P.U. of aqueous extract, an emulsion dose of 10,000 or 20,000 units was used.

New Cases.—A method of pre-treatment was employed for all individuals whose tolerances were unknown. These patients were given from two to four preliminary injections of aqueous extract at four- to seven-day intervals and were then given a multiple of the highest tolerated dose in emulsion form. In these cases no more than 25 times the highest aqueous dose was administered. Dose ranges varied from 500 P.U. to 20,000 P.U. for previously untreated patients.

Hemagglutination.—

Sera.—Samples of blood were obtained aseptically prior to injection and at varying intervals following injection during and following the 1960 pollen season. Sera were separated aspetically and stored at —20° C* until used.

Ragweed Extracts.—Twenty per cent w/v extracts of either defatted giant or short ragweed pollen extracts were prepared and used for conjugation with the red blood cells.

Bis-diazotized Benzidine Method.—A 0.1 per cent concentration of ragweed extract was coupled to a 2 per cent suspension of washed rabbit erythrocytes with a 1:15 dilution of B.D.B. All sera (previously heat inactivated for thirty minutes at 56° C) were absorbed with untreated aliquots of the erythrocytes used for conjugation with pollen extract. Serial twofold dilutions of such sera were incubated with 0.05 volumes of the B.D.B. ragweed-conjugated erythrocytes. Titers were recorded after eighteen hours as the reciprocal of the highest dilution of serum which gives a completely positive hemagglutination pattern. All sera were tested simultaneously in duplicate on at least two separate days. Similar titrations of standard pooled rabbit anti-ragweed sera with the B.D.B. treated red cells yielded titers of 1:100,000 or more.

Tannic Acid Method.—The tannic acid technique consisted of pretreatment of erythrocytes with a 1:20,000 dilution of tannic acid. Following suitable washing, such treated cells were coated with 0.1 per cent aqueous solutions of either giant or short ragweed. Such ragweed-coated, tannic acid-treated cells in 0.05 volumes were incubated with 0.5 ml volumes of sera dilutions exactly as done with the B.D.B. titrations.

Controls.—Controls during titrations consisted of the use of uncoated erythrocytes; the specific inhibition of all positive titers with ragweed extract; the use of known positive serum controls (including rabbit anti-

*This is the standard method used by serologists and immunologists and is dependent on the fact that storage in the deep freeze (-20° C) adequately preserves antibody and other protein moities of sera. There is little or no change in antibody titer in sera stored both in the freezer or the ice box for several months, as compared to sera at room temperature.

ragweed sera) in all tests; known negative serum controls, and testing with red cells coated with unrelated antigens (bovine gamma globulin, grass pollen extracts).

Clinical Evaluation.—All patients kept a detailed "log" of their symptoms and the amount of symptomatic medication needed during the pollen season. They were all seen at least three times during the season. The following scale was used to evaluate clinical results:

Clinical Responses	*Description*
Complete Relief	Asymptomatic
Excellent	Occasional symptoms
Moderate	Symptom-free most of the time
Fair	Relief less than half of the time
Poor	Very little freedom from symptoms

Any case having symptoms more than half of the time was considered a complete failure.

RESULTS

Clinical Results and Reactions.—Of the thirty-nine patients treated in this series with repository injections, thirty-four had good results as indicated by clinical relief during the season (Table I). However, the 1960 season was a relatively mild one in Philadelphia as far as pollen counts were concerned. The ragweed count was 488 pollen grains per square centimeter

TABLE I. CLINICAL RELIEF DURING SEASON

Previous Treatment	Clinical Results				
	Free	Excellent	Moderate	Fair	Poor
None	1	0	4	1	0
Perennial	3	9	5	1	0
Pre-seasonal	0	7	5	1	2
Total	4	16	14	3	2

Clinical results of thirty-nine patients during 1960 ragweed pollen season following repository injection of ragweed emulsion.

as opposed to the usual average of 1,000 pollen grains.[34] No delayed reactions, cysts, or draining sterile abscesses were observed. Three patients had mild "constitutional" reactions several hours following injection. All were relieved by the administration of oral antihistaminic agents and bronchodilator drugs. The reactions considered as constitutional consisted of any reaction other than local, and manifested by rhinorrhea, nasal blockage, sneezing, lacrimation, urticaria, or pruritus. Local swelling or inflammation at the site of injection was considered an immediate local reaction. Fourteen of these reactions occurred; three were severe; three were moderate, and eight were mild. The mild reactions were no more troublesome than the usual local reactions seen after the injection of aqueous extract. The severe and moderate local reactions remained swollen and inflamed for periods of one to seven days.

Hemagglutination Tests.—Each serum was tested for anti-ragweed antibodies by passive hemagglutination procedures using both the bis-diazotized benzidine method and the tannic acid method. Each of these procedures

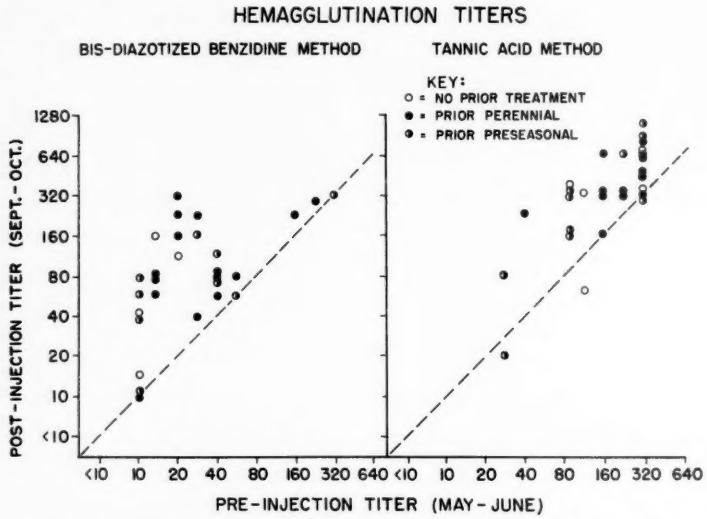

Fig. 1. Comparison of hemagglutination titers as measured by the B.D.B. and the tannic acid method of pre- to post-treatment sera.

TABLE II. COMPARISON OF B.D.B. METHOD WITH TANNIC ACID METHOD

Titer Change	B.D.B. Method		Tannic Acid Method	
	Number	Per Cent	Number	Per Cent
Increase:				
Two-fold	10	35	13	46
Four-fold	7	25	5	18
Eight-fold or greater	8	29	4	14
Same	3	11	5	18
Decrease	0	0	1	4

Comparative changes in hemagglutination titers of pre- to post-treatment sera of twenty-eight patients treated with ragweed emulsion. Rabbit red cells coated with short ragweed extract.

was used with rabbit erythrocytes, congugated with short ragweed pollen extract and with giant pollen extract.

Figure 1 indicates the change in hemagglutination titers of twenty-eight patients treated with ragweed repository emulsion and tested by the B.D.B. and tannic acid methods. There is a demonstrable rise in titer of the post-treatment sera as opposed to the pre-treatment sera as measured by either procedure with the use of short, ragweed-coated erythrocytes. However,

there is apparently little relationship between the titers measured by the tannic acid method and by the B.D.B. method (Table II).

The lack of correlation is further exemplified by a comparison of the B.D.B. titers with the tannic acid titers using either short ragweed or giant ragweed and sera from patients both prior to treatment and following treatment (Fig. 2). Except for the apparent overall rise in titers, there is no correlation among the titers using either of the hemagglutination methods and either of the ragweed extracts.

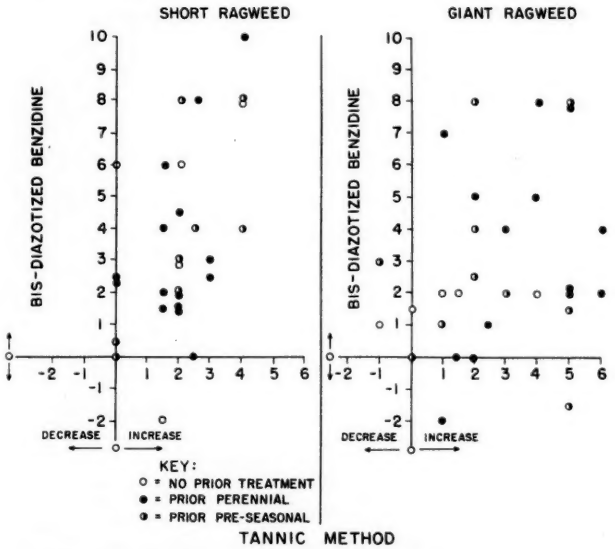

Fig. 2. Comparison of hemagglutination titers of sera from repository injected patients using either short or giant ragweed extract to sensitize rabbit red cells.

In many instances, there is a marked increase in titer as measured by the B.D.B. test, and a low or negligible change in titer in the same sera as measured by the tannic acid method (Fig. 3). There is no correlation or consistency between such changes in titer where identical serum dilutions were tested by the B.D.B. method, the tannic acid method, and the erythrocytes coated with either giant ragweed or coated with short ragweed extracts.

In all of these titrations, each individual serum gave reproducible titers when tested repeatedly by the same hemagglutination procedure. Thus, a single serum would give a reproducible hemagglutination titer when titrated by a given procedure (such as tannic acid procedure with giant ragweed extract). No correlation could be observed, however, between the various

titers obtained by any one of the hemagglutination procedures when compared to each other with the use of an individual serum. Controls were consistent within each procedure and gave reproducible results.

Comparison with Clinical Relief.—Hemagglutination titers and changes in such titers from pre- to post-treatment specimens indicated no correlation

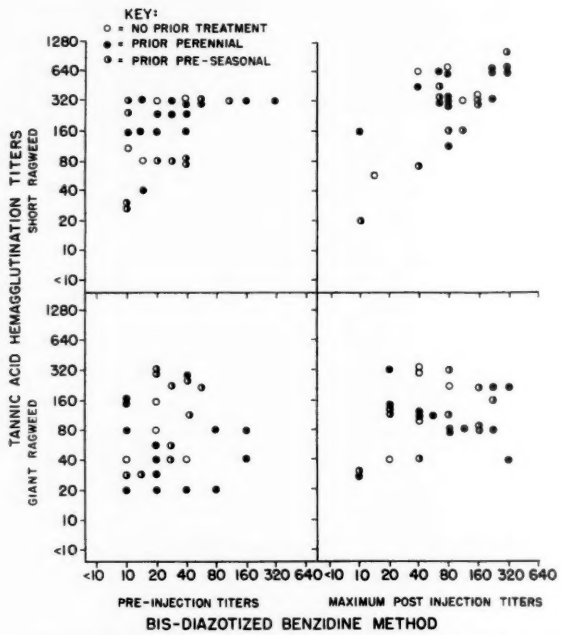

Fig. 3. Comparison of serum titers using B.D.B. or tannic acid methods.

TABLE III. MEAN TITERS AND MEAN TITER CHANGES

Clinical Response	Patients		Mean Peak Hemagglutinin Titers (Short Ragweed)					
			B.D.B. Method			Tannic Cell Method		
	Number	Per Cent	Pre-Treatment	Post-Treatment	Mean Increase	Pre-Treatment	Post-Treatment	Mean Increase
Free	1	4	10	10	0	160	160	0
Excellent	13	44	35	127	92	196	314	118
Moderate	11	4	30	114	84	299	447	148
Poor-fair	3	12	113	153	40	240	373	133

Comparison of clinical relief and mean peak titers of sera tested by B.D.B. and Tannic acid hemagglutination methods following repository emulsion ragweed treatment of hayfever patients.

with clinical relief. Table III indicates the mean titers and mean titer changes as measured either by the B.D.B. method or the tannic acid method in comparison to subjective degrees of clinical relief for each patient. Ex-

cept for the overall rise in titer of most subjects, there is no correlation between titers and relief or lack of relief of clinical symptoms. This is further exemplified by Figure 4 in which two-fold changes in titers are plotted in reference to clinical relief. No correlation can be noted. As an example, one patient had total relief and no change in titer, while others had variable symptomatic relief without any relationship to changes in titers. Again there is little correlation in titers as measured by either the B.D.B. or tannic acid method. Similarly, there was little relationship

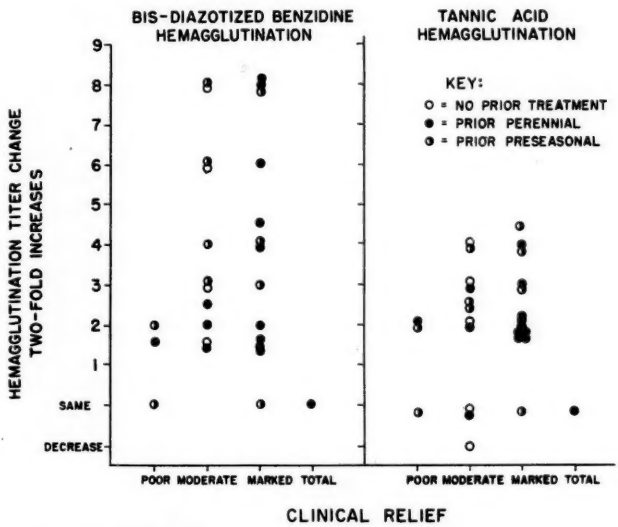

Fig. 4. Relationship of serum hemagglutination titer changes with clinical relief following repository injection.

exhibited between titers when giant ragweed was used as compared to short ragweed.

Effect of Prior Treatment.—In no case was there any evidence of a relationship between prior treatment with either hemagglutination titers or clinical relief following repository injection. As can be seen from the figures (Figs. 1-4), there is no apparent correlation between prior treatment (either pre-seasonal or perennial) or lack of prior treatment and hemagglutination responses.

Effect of Dosage.—Variation in dosages in the emulsions injected apparently has little effect on the overall final hemagglutination titer of each patient or on the change in titer. Figure 5 indicates some of the hemagglutination titer changes. Again, there is no correlation between change in

titer (as measured by the B.D.B. or tannic acid method) and the amount of ragweed extract in each emulsion preparation. There is no apparent correlation between such titers, emulsion dosage, and clinical relief of subjects.

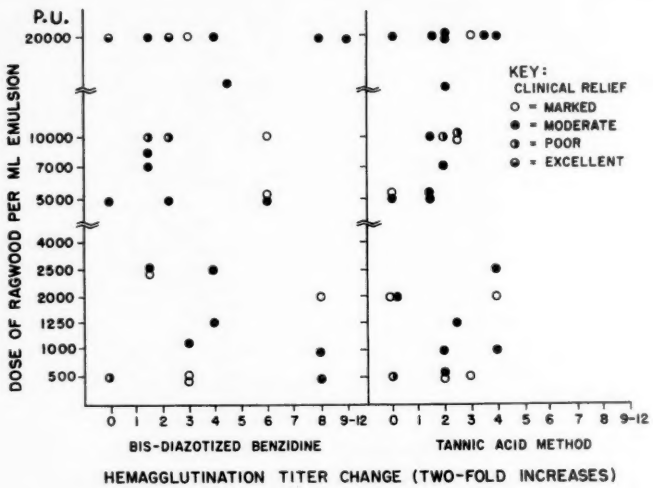

Fig. 5. Relationship of variation in ragweed dosage in emulsion with changes in hemagglutination titers of sera following repository injection.

DISCUSSION

There has been hope that *in vitro* serological procedures could be a quantitative immunologic procedure for detecting biologically significant anti-ragweed antibodies in the sera of allergic individuals.[23,27] There had been some discussion that such hemagglutination could detect not only "conventional blocking" antibody following therapy but even skin sensitizing antibody.[28] This expectation has not been confirmed by recent studies, including this one.

Arbesman has described the low specificity of the B.D.B. procedure, including a significant number of positive B.D.B. anti-ragweed titers in a series of sera obtained from Israel where there is no ragweed.[30] In the hands of Arbesman's group, the tannic acid hemagglutination method has been superior to the B.D.B. method in sensitivity, specificity, and reproducibility. However, others have pointed out some of the difficulties inherent in the tannic acid procedure, including the instability of the red-cell-antigen bond and the continued elution of antigen from such conjugated cells. Such elution results in inhibitory effects due to excess soluble antigen.[35] The firm chemical bonding with the B.D.B. method had given hopes that such a procedure would yield a more superior red-cell-antigen reagent. With the use of defined protein antigens, such procedures have

been quite useful and have extended the sensitivity of serological reactions many fold.

The use of such procedures with complex and unidentified pollen extract antigens has many innate problems. No defined antigen is known, and there is no information as to what constituent or constituents of the pollen extract is bound to the red cell. And, if many antigens are bound, what are the proportions? The difficulty of obtaining standardized sensitized red-cell ragweed conjugates is formidable. It could be suggested that the technical aspects of these procedures may be the cause of the apparent lack of correlation among titers. There is no evidence that the same ragweed pollen antigens are bound to erythrocytes when either the tannic acid or B.D.B. method is used.

In this study, reproducible results (within a two-fold dilution) could be obtained using a single serum specimen tested on various days with similarly prepared red cell conjugates. However, when red cells were treated with different preparations of ragweed extract (giant or short), or when the tannic acid method was compared to the B.D.B. method, titers were consistently varied. As indicated in the report, no correlation could be observed between such titers and any of the parameters studied. Except for the observation of a general increase in titers following repository injection, the only consistent observation is the lack of correlation.

Even if such lack of correlation could be entirely attributed to technical difficulties and the lack of a purified pollen antigen, it would seem that theoretical considerations should suggest that *in vitro* determination of anti-ragweed antibodies may have little correlation with clinical symptoms and therapeutic results. Regardless of whether conventional antibodies and/or skin sensitizing antibodies have anything to do with clinical relief or clinical symptomatology, it would seem that in an individual subject, the level of such antibodies in the circulation should vary continuously. It could be expected that the circulating threshold level of such antibody, needed either for possible therapeutic relief or for actual sensitivity, varies among individuals. Analogous to the findings of variable threshold amounts of antitoxin antibodies needed for clinical protection of individuals against bacterial toxins, it would appear that protective amounts should vary from individual to individual in this system. Once a threshold level of circulating antibodies necessary for protection has been reached, there should be little expectation that increased levels would give increased clinical protection. Thus, on theoretical grounds, there is little reason to suspect that the amount of circulating anti-ragweed antibody at any period of time should have any major correlation with the degree of clinical symptomatology or sensitivity.

In the case of delayed hypersensitivity phenomena and in suspected autoimmune syndromes, it has been suggested that the great variety of circulating antibodies against auto-antigens in the various disorders is a correlative

finding and has little to do with the actual immunopathology.[36] A closer correlation has been observed between skin sensitivity and such disorders than with circulating antibodies.

As far as the repository form of therapy is concerned, there is more detailed evidence of its clinical efficacy in other studies.[6,8,10] However, one should note the limitations in comparing the results of various investigators. These limitations are imposed because of the use of various methods of emulsification; various methods of treatment and injection; various methods of emulsion preparation; of dosages; of drug protection; and the like. However, these immunological studies do suggest strongly that there is a measurable response to ragweed among repository treated patients as detected by the appearance of circulating hemagglutinins. This response is comparable or greater than the responses observed in other series of repository or conventional aqueous ragweed injection treated patients.[33] Unfortunately, it appears from this study that there is essentially no correlation between such responses and any clinical or dosage levels. Thus, such responses must be considered as only another manifestation of immunological response to therapy without any determinable significance at the present time.

SUMMARY

1. Ragweed extract in the form of a water-in-oil emulsion has been given as a repository injection to a closely observed group of pollinotic patients for the treatment of hayfever.

2. Clinical relief from symptoms is similar to that following the usual aqueous multiple injections.

3. Anti-ragweed antibodies have been measured in pre- and post-treatment sera by hemagglutination procedures. Both the bis-diazotized benzidine method and the tannic acid method with rabbit erythrocytes conjugated to giant ragweed extract and to short ragweed extract were used.

4. There is no correlation of serum antibody titers obtained with any of the hemagglutination procedures used.

5. No correlation was observed between hemagglutination titers and clinical effects or dosage of repository injection.

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REFERENCES

1. Brown, E. A.: The prevention of reactions to repository injection treatment. Ann Allergy, 15:499, 1957.
2. Brown, E. A.: The treatment of ragweed pollinosis with a single, annual emulsified injection, II. Ann Allergy, 16:281, 1958.
3. Brown, E. A.: The treatment of tree pollen sensitivity with single annual injections of emulsified extract, III. Ann Allergy, 16:353, 1958.
4. Brown, E. A.: Treatment of grass pollinosis with a single annual injection of emulsified extract, IV. Ann Allergy, 16:510, 1958.
5. Brown, E. A.: The treatment of pollinosis by means of a single annual injection of emulsified extract, V. Ann Allergy, 17:34, 1959.

6. Brown, E. A.: The treatment of allergy to ragweed pollen by means of a single annual injection of emulsified pollen extract, VI. Ann Allergy, 17:352, 1959.
7. Loveless, M. H.: Application of immunologic principles to management of hayfever including preliminary report in the use of Freund's adjuvant. Amer J Med Sci, 214:559, 1957.
8. Loveless, M. H.: Repository injection of pollen allergy. J Immunol, 79:68, 1957.
9. Feinberg, A. R., Feinberg, S. M., and Fisherman, E. W.: Repository antigen injections, preparation and therapy. J Allergy, 31:433, 1960.
10. Furstenberg, F. F.: The repository method for the treatment of ragweed pollinosis (1959), a cooperative study involving 1,218 patients. Ann Allergy, 18:866, 1960.
11. Brown, E. A.: The treatment of pollinosis. Rev Allergy, 12:584, 1958.
12. Coca, A. F. and Grove, E. F.: Studies on hypersensitivity, XIII. J Immunol, 10:445, 1925.
13. Hampton, S., Johnson, M. C., Alexander, H. L., and Wilson, K. S.: Detection of the "thermostabile" antibody by means of the precipitin reaction. J Allergy, 14:227, 1943.
14. Bukantz, S. C., Johnson, M. C., and Hampton, S.: Qualitative immunologic studies with allergens. J Allergy, 20:1, 1949.
15. Cavelti, P. A.: Complement fixation studies in Allergy. J Allergy, 21:532, 1950.
16. Portnoy, J., and Sherman, W. P.: Complement fixation studies in ragweed allergy. J Allergy, 25:229, 1954.
17. Follensby, E. M.: The applicability of *in vitro* tests in the measurement of neutralizing antibody for ragweed pollen extract. J Allergy, 29:103, 1958.
18. Cohen, N. B., and Weller, R. R.: Precipitins in the sera of patients with clinical allergy. J Allergy, 12:242, 1941.
19. Swinford, B., and Honlehan, R.: Antibodies to ragweed extract. J Allergy, 18:190, 1947.
20. Coombs, R. R. A., Howard, A. N., and Mynors, L. S.: A serological procedure theoretically capable of detecting incomplete or non-precipitating antibodies to soluble protein antigens. Brit J Exp Pathol, 34:525, 1953.
21. Boyden, S. V.: The adsorption of proteins on erythrocytes treated with tannic acid and subsequent hemagglutination by antiprotein sera. J Exp Med, 93:107, 1951.
22. Orlans, E. S., Rubenstein, L. J., and Marrack, J. R.: Detection of antibodies in the serum of patients suffering from hayfever. Acta Allergol, 6:33, 1953.
23. Feinberg, R. J., Davidson, J. D., and Flick, J. B.: The detection of antibodies in hayfever by means of hemagglutination. J Immunol, 77:279, 1956.
24. Pressman, D., Campbell, D. H., and Pauling, L.: The agglutination of intact azo-erythrocytes by antisera homologous to the attached group. J Immunol, 44:101, 1942.
25. Stavitsky, A. B., and Arquilla, E. R.: Micromethods for the study of proteins and antibodies, III. J Immunology, 74:306, 1955.
26. Sehon, A. H., Gordon, J., and Rose, B.: Antibody-like factor in serums of ragweed sensitive individuals shown *in vitro*. Science, 125:597, 1957.
27. Gordon, J., Rose B., and Sehon, A. H.: Detection of "Non-precipitating" antibodies in sera of individuals allergic to ragweed pollen by an *in vitro* method. J Exp Med, 108:37, 1958.
28. Sehon, A.: The detection and nature of non-precipitating antibodies in allergic sera, in *Mechanisms of Hypersensitivity*, p. 61. Boston: Little, Brown, and Company, 1959.
29. Sehon, A.: Different types of antibodies produced by allergic individuals depending on route of immunization, in *Mechanisms of Antibody Formation*, p. 79. Prague, 1960.
30. Arbesman, C. E., Rose, N. R., Kantor, S. Z., and Beede, R. B.: Immunologic studies of ragweed-sensitive patients, I. J Allergy, 31:317, 1960.
31. Arbesman, C. E., Rose, N. R. and Kantor, S. Z.: Immunologic studies of ragweed-sensitive patients, II. J Allergy, 31:333, 1960.
32. Arbesman, C. E., Kantor, S. Z., Rapp, D., and Rose, N. R.: Immunologic studies of ragweed-sensitive patients, III. J Allergy, 31:342, 1960.
33. Criep, L. H., and Friedman, H.: Immune responses to repository injections of ragweed antigens. Fed Proc, 19:598, 1960.
34. Blumstein, G., and Spiegelman, J.: Annual Pollen Surveys (unpublished).
35. Feinberg, R. J., and Flick, J. A.: Elution of pollen antigens from tannic acid treated erythrocytes. Proc Soc Exp Biol Med, 96:71, 1952.
36. Waksman, B. H.: Delayed hypersensitive reactions; a growing class of immunologic phenomena. J Allergy, 31:468, 1960.

UNUSUAL EXTRA-RESPIRATORY MANIFESTATIONS OF POLLEN ALLERGY

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THIS PAPER presents experiences with certain unusual extra-respiratory manifestations of pollen allergy including chronic urticaria, headache, gastro-intestinal disturbances, unclassified arthritis, gouty arthritis, genito-urinary disturbances, fatigue and toxemia, oral disturbances, myalgia, neuralgia and purpura. Our experiences with atopic dermatitis,[1] chronic ulcerative colitis,[2,3] and regional enteritis[4] as a result of pollen allergy alone and with food allergy have been reported in other communications.

Moore[5] and Kahn[6] have described extra-respiratory symptoms of pollinosis. These authors stressed the frequently encountered psychological and neurologic disturbances. In Moore's group of 300 adults and twenty-two children, extra-respiratory symptoms appeared both in the presence and in the absence of classical hay fever and asthma but were usually of secondary clinical importance. They presented a review of the literature.

In our group of thirty-seven patients, the extra-respiratory symptoms predominated. Other allergic manifestations, when present, were of secondary importance. Pollen allergy was suspected through careful history taking. In most instances, the seasonal exacerbations were obvious to the physician. Occasionally, however, initial patient interviews were misleading, especially when symptoms were perennial with seasonal exacerbations. In these patients, symptoms were attributed erroneously at first to probable food, drug, or environmental inhalant sensitivity. Only after several months of observation did the exacerbations during pollen seasons become obvious to physician or patient, thus establishing the correct diagnosis. In most cases there were confirmatory positive scratch and/or intradermal skin tests to seasonal pollens. Skin tests were equivocal or negative in seven patients (16 per cent). In these patients, the diagnosis was established on the basis of the clinical history and response to hyposensitization therapy. All patients were completely tested by the scratch method initially and subsequently with intradermal tests by serial dilution titrations.

In all patients, treatment consisted of perennial hyposensitization over periods of from one and one-half to four years, with polyvalent pollen extracts containing all-important pollens in their residential area fortified with seasonal pollens selected on the basis of skin tests and history. Maximum dilutions of pollen extracts attained varied from 1:10,000 to 1:100. Initial dilutions used in hyposensitization therapy were frequently consider-

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TABLE I. URTICARIA

Patient	Age	Duration (Years)	Seasonal Incidence	Pollen Skin Tests		Perennial Hyposensitization (Years)	Result	Remarks
				Spring	Fall			
1	60	2	Mar-Oct	4+	3+	2	Relief	
2	42	2	Mar-Aug	6+	4+	1½	Relief	Associated spring hay fever and asthma
3	10	2	Apr-July	2+	0	2	Relief	
4	32	1	Mar-June	5+	0	3	75% relief	
5	38	2	Mar-June	+	0	3	80% relief	
6	33	5	June-Oct	2+	2+	2	95% relief	
7	43	4	Apr-Oct	2+	4+	3	Relief	
8	6	2	May-Oct	+	+	2	50% relief	

ably weaker. Injections were administered at intervals of from three to seven days. In several patients, symptoms were frequently reproduced by overdoses of pollen extract, administered out of the pollen season. Complete relief of symptoms was obtained in 81 per cent of patients, and partial relief in 19 per cent. The degree of relief achieved did not seem related to the severity of symptoms or to the duration of therapy.

In a minority of cases (22 per cent) there were additional manifestations of pollen allergy and/or associated food allergy. As stressed above, these were of secondary importance.

CLINICAL NOTES AND DISCUSSION

Urticaria (Table I).—Of the eight patients studied, only one (Case 2) presented other allergic symptoms. Four patients obtained complete relief. In four patients, relief varied from 50 per cent to 95 per cent. In cases five and eight, skin reactions were equivocal or negative.

Headache (Table II).—In all six patients, there were no additional manifestations of allergy. Symptoms were entirely seasonal in four patients. Two patients, represented by Case 1 and Case 5, complained of perennial

TABLE II. HEADACHE

Patient	Age	Duration (Years)	Seasonal Incidence	Pollen Skin Tests		Perennial Hyposensitization (Years)	Result	Remarks
				Spring	Fall			
1	32	3	Perennial spring aggravation	6+	3+	1½	Relief	Unilateral, associated spring hay fever
2	31	13	Sept-Dec	0	+	1	Relief	Unilateral with rhinorrhea and lacrimation (histamine-like)
3	43	20	Mar-Aug	3+	0	1½	Relief	Bilateral
4	31	19	July & Aug	6+	3+	1	Relief	Unilateral with ipsolateral facial edema
5	38	20	Perennial with summer aggravation	1+	1+	1	Relief	Bilateral with nausea, vomiting, dimness of vision
6	36	5	May-Aug	0	0	4	Relief	Unilateral with ipsolateral lacrimation, rhinorrhea, sneezing

TABLE III. GASTROINTESTINAL

Patient	Age	Duration (Years)	Seasonal Incidence	Pollen Skin Tests		Perennial Hyposensitization (Years)	Result	Remarks
				Spring	Fall			
1	46	22	Feb-June	0	0	2	Relief	Mucous colitis
2	49	3	Mar-June	0	0	1	Relief	Duodenal ulcer, constipation, gas, indigestion
3	45	20	Mar-June Aug-Oct	6+	4+	2	Relief	Pressure, gas, burning, constipation, mild perennial symptoms
4	50	3	Mar-July	1+	1+	2	75% relief	Abdominal soreness, pain
5	36	5	Mar-Oct	1+	1+	3	Relief	Abdominal pain, vomiting, diarrhea, postnasal discharge with choking
6	55	15	Mar-Oct	3+	3+	2	Relief	Abdominal pain, cramps, diarrhea

headache with definite seasonal exacerbations. In these patients, complete relief was also obtained. Case 2 presented a clinical picture of erythromelalgia. This patient had received histamine hyposensitization therapy previously without benefit. Skin tests were negative or equivocal in this patient and also in the patient represented by Case 6.

Gastrointestinal (Table III).—Symptoms varied widely in this group of six individuals and included abdominal soreness, bloating, flatus, belching, mucous colitis, cramping, diarrhea and constipation alone or in various combinations. The patient in case 2 presented herself with a proven duodenal ulcer which completely healed under pollen hyposensitization. Skin tests were negative in this individual and also in the patient noted as Case 1. Complete relief of symptoms was obtained in all but one patient, who was, however, greatly relieved. We have excluded from this communication our patients in whom pollen allergy has been proven to be the etiologic factor in chronic ulcerative colitis and regional enteritis.[2,3]

Arthritis (Table IV).—Four patients with seasonal unclassified joint disease were encountered. X-ray findings were normal in all. Sedimentation rates were initially elevated in two patients returning to normal during therapy. In Case 2, omission of regular hyposensitization injections resulted in aggravation of joint symptoms during the first year of treatment. The patient represented by Case 3 suffered from seasonal hay fever and gastrointestinal food allergy. Her symptoms were markedly worse in the San Joaquin Valley during the spring and fall and less severe near the Pacific Ocean prior to treatment.

Genito-urinary (Table IV).—In the patient represented by Case 1, both pollen and food allergy were responsible for attacks of ureteral colic and the presence of increased mononuclear cells in the urinary sediment. Initial control of her food allergy did not control the seasonal exacerbations due

TABLE IV. ARTHRITIS AND GENITO-URINARY SYMPTOMS

Patient	Age	Duration (Years)	Seasonal Incidence	Pollen Skin Tests		Perennial Hyposensitization (Years)	Result	Remarks
				Spring	Fall			
Arthritis								
1	69	20	Apr-Aug	3+	0	2	Relief	Stuffiness, soreness neck and shoulders, severe, x-rays negative
2	25	5	Perennial with aggravation March-June	1+	2+	2	Relief	If injections omitted joints flared during first year
3	29	3	Mar-Oct	4+	1+	4	Relief	Hand arthritis worse in valley, hay fever, G-I allergy
4	28	4	Perennial with aggravation spring and fall	2+	4+	4	Relief	Polyarthritis hay fever
Genito-Urinary Symptoms								
1	52	5	Perennial with spring aggravations	1+	0	2	Relief	Ureteral colic relieved by Epinephrine, mono-nuclear cells in sediment. Associated food allergies
2	55	4	March-Oct	3+	1+	1½	90% relief	Pruritus vulvae

to pollen sensitivity. During hyposensitization treatment, overdoses of antigen promptly reproduced symptoms. The patient noted in Case 2 complained only of pruritus vulvae present from March through October. She experienced almost complete relief.

Gout (Table V).—One patient was encountered with gouty arthritis. Classical radiological findings and hyperuricemia were present. Symptoms occurred only from March through July for two years. Therapy gave complete relief.

Fatigue and Toxemia (Table V).—The severe fatigue, dopiness and irritability experienced by these two patients from March through October, Case 1, and March through July, Case 2, were due entirely to pollen sensitivity. Both obtained complete dramatic relief of symptoms with hypo-

TABLE V. GOUT, FATIGUE AND TOXEMIA, AND ORAL SYMPTOMS

Patient	Age	Duration (Years)	Seasonal Incidence	Pollen Skin Tests		Perennial Hyposensitization (Years)	Result	Remarks
				Spring	Fall			
Gout								
1	44	2	Mar-July	4+	2+	2	Relief	Great toe; hay fever Mar-Oct
Fatigue and Toxemia								
1	25	5	Mar-Oct	4+	2+	1	Relief	Fatigue, dopiness
2	35	5	Mar-July	4+	1+	2	Relief	Fatigue, irritability, severely; hay fever, asthma mildly
Oral Symptoms								
1	43	8	Mar-Oct	3+	2+	2½	Relief	Fire-like burning perennial—worse spring and fall
2	29	4	Mar-June	+	+	2	Relief	Sore mouth, coated tongue, exfoliation of hands

TABLE VI. MYALGIA, NEURALGIA AND PURPURA

Patient	Age	Duration (Years)	Seasonal Incidence	Pollen Skin Tests		Perennial Hyposensitization (Years)	Result	Remarks
				Spring	Fall			
Myalgia								
1	43	5	Apr-Oct	2+	2+	4	Relief	Spring and fall hay fever, mild
2	13	3	Mar-Sept	3+	0	3	Relief Myalgia —100% Asthma —80%	Suspected of polio twice, asthma May and June
Neuralgia								
1	29	3	June-Nov	0	2+	3	Relief	Severe right facial pain, 5th cervical
2	38	3	Mar-Oct	0	0	2	Relief	C5, 6, 7, ACTH gave relief prior to hyposensitization
Purpura								
1	29	6	Mar-Apr	+	0	3	Relief	Purpura annularis telangiectodes (Majocchi's disease)

sensitization therapy alone. Allergic toxemia and fatigue due to food allergy has been previously described by one of us.[7]

Oral symptoms (Table V).—Patient 1 had suffered from severe fire-like burning of the mouth for eight years perennially, with distinct exacerbations yearly from March through October. Patient 2, in addition to experiencing severe mouth soreness and redness of oral and gingival mucous membranes, had exfoliation of the skin of the hands, both present from March through July only for a period of four years. In both patients, complete relief was obtained.

Myalgia (Table VI).—Both patients complained of generalized severe muscle aching from April through October and from March through September. Patient 2 had been suspected of having poliomyelitis on two occasions. He also suffered from asthma in May and June. In this patient, hyposensitization therapy completely relieved the myalgia but resulted in only 80 per cent relief of asthma.

Neuralgia (Table VI).—In patient 1 there was severe right facial and fifth cervical root pain present from June to November for three years. Patient 2 had experienced excruciating right cervical neuralgia involving roots 5, 6 and 7. Questionable changes of the corresponding vertebral foramina were noted by x-ray. Corticotropin had afforded relief prior to our therapy. In this patient, skin tests were negative. Symptoms completely disappeared out of her pollen seasons. In both cases relief was complete with treatment.

Purpura (Table VI).—This patient presented the clinical picture of purpura annularis telangiectodes (Majocchi's Disease). Lesions had recurred in March and April only for six years. Overdoses of pollen antigens

out of her pollen season reproduced lesions on four occasions. Complete relief was obtained with maximum antigen strength of 1:100.

CONCLUSIONS

1. Unusual extra-respiratory tract manifestations of pollen allergy in thirty-seven patients are described. In all patients, extra-respiratory symptoms were of prime importance, and other manifestations of allergy when present were of secondary importance.

2. Symptoms included urticaria, headache, varied gastrointestinal disturbances, arthritis, genito-urinary complaints, gouty arthritis, fatigue and toxemia, oral disturbances, myalgia, neuralgia and purpura.

3. Negative or equivocal scratch and/or intradermal tests to pollen were obtained in seven patients (16 per cent).

4. In all patients, the diagnosis was suspected through careful history taking, stressing the importance of seasonal exaggeration of symptoms as an indication of pollen allergy. If symptoms were perennial with seasonal exacerbations, food, drug, or environmental inhalant sensitivity was erroneously suspected initially. In such patients, exacerbations coincident with pollen seasons became apparent only after several months of observation.

5. Perennial hyposensitization with polyvalent extracts prepared according to areas of residence and fortified with appropriate seasonal pollens was given to all patients for periods of from one-half to four years. Maximum dilutions of pollen extracts attained varied from 1:10,000 to 1:100. Initially employed dilutions were frequently considerably weaker.

6. Clinical symptoms were frequently reproduced by over-dosage of antigen given out of the pollen season.

7. Twenty-two per cent of patients suffered from other allergic manifestations attributed to pollen and/or food allergy. These were of secondary importance.

8. Complete relief in 81 per cent of cases and partial relief (50 to 95 per cent) in 19 per cent was obtained.

9. The degree of relief obtained was not related to the severity of symptoms or to the duration of therapy.

REFERENCES

1. Rowe, Albert Jr.: Atopic dermatitis due to sensitivity to pollen. Calif Med, 91:341-343, 1959.
2. Rowe, Albert H., Rowe, Albert, Jr.: Chronic ulcerative colitis and regional enteritis—their allergic aspects. Ann Allergy, 12:387-402, 1954.
3. Rowe, Albert H., Rowe, Albert, Jr., Uyeyama, Kahn: Chronic ulcerative colitis due to pollen allergy with six case reports. Acta Med Scand, 152:139 fasc II, 1955.
4. Rowe, Albert H., Rowe, Albert, Jr.: Chronic ulcerative colitis and regional enteritis responding to anti-allergic therapy. Gastroenterologia, 91:353, 1959.
5. Moore, Merle W.: Extra-respiratory tract symptoms of pollenosis. Ann Allergy, 16:152, 1958.
6. Kahn, I. S.: Pollen toxemia in children. JAMA, 88:241, 1927.
7. Rowe, Albert H.: Allergic toxemia and fatigue. Ann Allergy, 8:72, 1950.

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ANIMAL TOXICITY EVALUATION OF DRAKEOL-ARLACEL MIXTURES USED FOR ALLERGENIC EXTRACT EMULSIONS

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WATER-IN-OIL emulsions of allergenic extracts are currently being evaluated clinically for their effectiveness in allergic diseases. Brown,[1-9] and Loveless,[11-13] have established the usefulness of emulsified extracts in therapy.

The basic immunological concepts of emulsion therapy have been demonstrated by Freund and others.[10,14]

This paper is concerned with the investigation of the potential toxic properties of the emulsification vehicle which consists of a mixture of Light Mineral Oil (Light Liquid Petrolatum) NF XI (Drakeol 6) and specially treated Mannide mono-oleate (Arlacel A).

In each of the experiments, a sterile mixture of Drakeol-Arlacel* was used. This product has the trade name of Daroil-10. Tests for safety by parenteral and topical applications were performed in experimental animals. The tests included acute toxicity in mice, subacute and chronic toxicity in guinea pigs, local irritation and sensitization properties and chronic toxicity of a prolonged injection site in rabbits.

METHODS AND FINDINGS

General—In all toxicity tests employing mice, inbred strains of mice were used to assure uniformity of response within the group and as a comparison with controls. All animals used were normal, adult and of both sexes. Prior to each toxicity test, the animals were observed under quarantine conditions for a period of ten days. Bacterial determinations for freedom from latent respiratory and enteric infections were done. In each toxicity test, groups were designed so that there was an equivalent distribution on the basis of age, sex and weight.

1. *Acute Toxicity in Mice.*—Four groups, eight mice each of strain C57BL/6, were used and treated with the following doses and routes:

Group I	0.25 ml	Daroil-10, intraperitoneally
Group II	0.25 ml	Daroil-10, subcutaneously
Group III	0.50 ml	Daroil-10, intraperitoneally
Group IV	0.50 ml	Daroil-10, subcutaneously

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*Drakeol-Arlacel, a sterile mixture of 10 per cent Arlacel A, specially treated, and 90 per cent Light Liquid Petrolatum, NF XI (by volume) manufactured by Center Laboratories, Inc., Port Washington, N. Y., for investigational use.

The dose employed for each individual mouse was the same dose intended for human injection. Therefore, on a body-weight basis, the dose represented a considerable increase over that administered to humans.

The observations made were: general behavior and appearance; reaction to sound and touch stimulus; local reaction at the site of subcutaneous injection. The animals were examined every two hours during the first twelve hours and twice daily thereafter.

Four mice per group were sacrificed twenty-four hours after the injection, and the remaining four mice per group were sacrificed three days after the injection. Complete gross necropsy was performed with special scrutiny of the injection sites. Tissues for histologic examination were prepared from the injection site. Liver, spleen, kidney, small intestine, lungs, and regional inguinal or axillary nodes were taken if enlargement was noted.

Findings.—All animals receiving intraperitoneal injections had mild, transient abdominal discomfort evidenced by contraction of the abdominal musculature for approximately two to five minutes. No local reaction to the subcutaneous injection was noted. None of the injected mice exhibited any untoward symptoms or local reactions thereafter. At necropsy, a mild hyperemia was noted around the emulsion depot subcutaneously. The animals injected intraperitoneally showed no abdominal abnormalities; the emulsion was diffusely dispersed in the abdomen. There was no evidence of irritation, edema, or other signs of any toxic response. The animals were immediately subjected to autopsy and examined grossly for any evidence of demonstrable gross organ changes. None was found. All abdominal and thoracic organs were normal in appearance, size and position.

The viscera were removed. Blocks of tissue from each of the major structures such as the liver, the spleen, kidneys, pancreas, adrenals and gastrointestinal tract, as well as the heart and lungs and the subcutaneous tissue at the site of injection—in the case of subcutaneously injected animals—were fixed in 10 per cent formalin solution for subsequent sectioning. They were then stained with hematoxylin-eosin.

There were no histopathologic changes observed in any of the viscera which in any way could be attributed to this material, except for the ordinary amount of local inflammatory cellular infiltration (polymorphonuclear leukocytes) in the subcutaneous fat at the site of injection. There were no appreciable differences observed in the tissues of the animals sacrificed at twenty-four or seventy-two hours.

From the combined gross and microscopic examination of the tissues, it was concluded that there was no evidence of toxicity.

2. *Acute Toxicity on Topical Application to Open Wounds.*—The general procedures for animal selection were employed as given above. Groups of ten mice each were cleanly shaven on the dorsum and numbered. Under conditions of surgical asepsis, two wounds, each approximately 7 mm in

diameter and involving tissue down to the dorsal musculature, were created simultaneously on the dorsum of each mouse. A specially designed surgical skin biopsy instrument was employed which institutes two equal wounds simultaneously. The left wound served as an untreated control.† Daroil-10 was instilled into the right wound on each mouse with a 27-gauge needle. The sample was also injected intracutaneously into the skin at three points around the periphery of the wound.

Observations for local irritation, edema and necrosis were made during the remainder of the day of wounding, and twice daily thereafter until the animals were sacrificed. Each observation compared the status of the treated right wound and the untreated control left wound as well as the wound reactions between mice.

A second group of ten mice, each similarly prepared with wounds, were treated as above. During the first day, however, 0.01 ml of Daroil-10 was instilled into the right wound three times. Of the sample, 0.01 ml was instilled daily on the second through the fifth day. Five mice were sacrificed on the fifth day after having been wounded six to eight hours subsequent to the instillation of the last dose. The mice in the first group which received only one dose were also sacrificed on the fifth day after wounding. The remaining five mice in each group were sacrificed on the ninth day.

Findings.—After single injection of Daroil-10 in wounds, findings were as follows:

There was no evidence of any irritation or other untoward reaction in the treated wounds as compared with the control wounds on each animal in both groups. Except for the disturbance of the wound by repeated daily instillations in the second group, both the treated and untreated wounds appeared to heal uneventfully at approximately the same rate. Gross necropsy on both the fifth and ninth days did not reveal any evidence of damage either in the wound area or in any of the internal organs. Sections for histologic examination were taken of the control and treated wounds, lung, liver, spleen, kidney, and small intestine of each mouse. The wounds were prepared for histologic examination of interrupted serial sections through the wound area. Histologic evaluation of each of the wounds was made on coded slides. Control and treatment wounds could not be distinguished. Microscopic evaluation was based on: (a) degree of cellularity: exudation, leukocytes, edema, hemorrhage, (b) amount of collagenization, (c) amount and compactness of granulation tissue: new capillaries and stroma, (d) degree and extent of epithelialization: from no cover to completely covered, (e) degree of fibrosis: fibroplastic proliferation representing final stage of healing.

†Several years of experience by Drs. Molomut and Smith, of the Waldemar Medical Research Foundation, Port Washington, N. Y., with this technique in evaluating the stimulating or deterrent effects of innumerable agents has resulted in the standardization of the procedure and the use of these particular time intervals as providing completely satisfactory and valid quantitative data.

Each of these factors was graded on a 1 to 4 basis and recorded. These data were then decoded. There was no evidence of toxicity, irritation, or interference with the normal process of wound healing, as compared with control untreated wounds. In fact, there seemed to be some evidence, on the basis of histologic evaluation, that fibrous tissue repair seemed to be slightly more advanced in the treated wound than in the control wound on the same animal.

Microscopic Histologic Evaluation (after repeated injections of Daroil-10 in wounds).—The animals receiving multiple injections of Daroil-10 were sacrificed as follows: five at the end of five days and the remaining five at the end of nine days. As in the case of the acute toxicity with single injection, no irritative phenomena were noted either grossly or microscopically. There was no evidence of visceral damage involving heart, lungs, liver, spleen or kidneys. There was no delay caused in the healing time on a quantitative scale basis as above described. Treated lesions healed more rapidly than control untreated wounds.

There was no evidence of toxicity as measured by rate of normal tissue regeneration.

3. *Chronic Toxicity Test in Guinea Pigs.*—Six albino guinea pigs (three male, three female), weighing 250 to 300 grams, were cleanly shaven on both sides from the axillary to the inguinal region. Subcutaneous injection of 0.1 ml of the sample Daroil-10 was made in two sites (total dose 0.2 ml) one on the left side and one on the right side beginning at the axillary region. This dose was repeated weekly for a total of six weeks, each injection being given in another site in a caudal direction, so that, by the sixth injection, a line of injection sites on both sides of each animal had been made. Observations were made daily for local reactions. Ten days after the last injection, a final intracutaneous injection was made on each pig and observed for accelerated response. Twenty-four hours after this last injection, the animals were sacrificed. Gross necropsy was performed, and tissue sections were taken of injection site, liver, spleen, kidney, and small intestine.

None of the injection sites demonstrated sensitization or toxicity other than the formation of small granuloma typical of subcutaneous oil depot injections. There was no heightened or accelerated response to the intracutaneous challenge injection. Gross necropsy did not reveal any unusual or abnormal findings in the internal organs.

Microscopic Histologic Evaluation.—Examination was made of the liver, spleen, kidneys, gastro-intestinal tract, pancreas and adrenal glands. In the liver, no changes were observed other than scattered areas of granular degeneration. The latter bears no relation to the treatment. The kidneys, gastro-intestinal tract, pancreas and adrenal glands were normal. In the spleen, there was moderate hyperplasia of both the lymphoid and reticular

elements in four of the six animals. Whether this might possibly be the result of absorption of the lipid material from the local site of inoculation is doubtful, especially since there was no vacuolization to indicate the presence of fat.

No subacute or chronic changes of a pathologic significance were observed in any of the organs taken from this group of animals.

4. *Chronic Toxicity and Sensitization Test in Rabbits.*—Three 1500 gm rabbits were cleanly shaven on both lateral abdominal surfaces and injected intracutaneously with the following:

Left Side: Three sites with 0.02 ml Daroil-10 approximately 2 centimeters apart.

Right Side: One site with 0.02 ml Drakeol-6, one site with 0.02 ml Arlacel A, and one site with 0.02 ml 0.85% saline.*

The injection sites were examined for evidence of irritation or toxicity immediately after the injection. Fourteen days later, each rabbit was challenged with an intracutaneous injection of Daroil-10 and observed for sensitization response.

In addition, each rabbit was injected with 0.2 ml Daroil-10 deep intramuscularly into the right and left hind limbs. Sixty days later, under nembutal anesthesia, muscle biopsies were surgically taken from the intramuscular injection sites and prepared for histologic examination.

There was no evidence of local toxicity or sensitization in the intracutaneous injection sites described above. The local area contained typical oil depot granulomas which were slowly absorbed. Gross and microscopic examination of biopsy sections of the muscle injection sites taken under anesthesia revealed no evidence of irritation or tissue damage, and the injected sample had been completely absorbed from the injection sites.

Daroil-10 did not produce any local toxic response or sensitization in the skin or muscle of rabbits. Complete absorption, without residual damage, of the injected sample was observed.

SUMMARY

1. The mixture of Drakeol-6 and Arlacel-A (Daroil-10) was tested for gross and microscopic evidence of toxicity in mice, guinea pigs and rabbits by topical application and single and repeated parenteral injections.

2. There was no evidence of toxic response, employing human bodyweight equivalent doses, on wound healing and on subcutaneous and intramuscular injection sites.

3. There was no evidence of systemic toxic reaction and, after sixty days,

*Drakeol-6 (brand of Light Liquid Petrolatum, NF XI manufactured by Pennsylvania Refining Co.)

Arlacel-A (specially treated brand of Mannide mono-oleate, manufactured by Atlas Powder Co.)

there was no residual tissue damage or presence of the injected material in deep intramuscular sites in rabbits.

It is concluded, therefore, that Daroil-10 in the dosage employed (equal to the human dose) is non-toxic when employed locally and systemically.

REFERENCES

1. Brown, E. A.: Rev Allergy 12:584, 1958.
2. Brown, E. A.: Ann Allergy 15:499, 1957.
3. Brown, E. A.: Ann Allergy 16:281, 1958.
4. Brown, E. A.: Ann Allergy 16:353, 1958.
5. Brown, E. A.: Ann Allergy 16:510, 1958.
6. Brown, E. A.: Ann Allergy 17:34, 1959.
7. Brown, E. A.: Ann Allergy 17:358, 1959.
8. Brown, E. A.: Rev Allergy 13:803, 1959.
9. Brown, E. A.: Ann Allergy 18:1200, 1960.
10. Freund, J. and McDermott, K.: Proc Soc Exp Biol Med 49:548, 1942.
11. Loveless, M. H.: Am J Med Sci 214:559, 1947.
12. Loveless, M. H.: J Immunol 79:68 (July) 1957.
13. Loveless, M. H.: J Amer Med Wom Ass 15:955 (Oct.) 1960.
14. Salk, J. E., Bailey, M. L., and Laurent, A. M.: Amer J Hyg 55:439, 1952.

SIGNS OF PLEURISY

The signs of simple pleurisy are quite clear. They are fever invariably present because of the vicinity of the heart, secondly, stabbing pain under the ribs, since the membrane is shaggy, and often this is not apparent except when breathing. And sometimes there is tension with stabbing; sometimes it is very great. Tension signifies great extent and stabbing, great severity in its penetration. The third sign is difficulty of breathing or intercompression caused by affusion, also by the shallowness and frequency of breathing. The fourth is a saw-like pulse. The difference in the usual condition of the pulse is increased by the want of strength and the extent of the cause. The fifth sign is cough; sometimes the cough at the beginning of this illness is dry and later with sputum; and whenever the cough is with sputum at the beginning, it is a matter for congratulation. The cough, however, does not occur unless the lungs are affected by its proximity.—AVICENNA, 980-1037, *Canon of Medicine*, Book III, Tract IV.

VISUALIZATION OF THE FATE OF INJECTIONS OF WATER-IN-OIL EMULSIONS BY MEANS OF RADIOPAQUE MEDIA, II

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WHEN A SERIES of clinical observations demonstrated the feasibility of the treatment of pollinosis by means of a single annual injection of emulsified extract,[1] further studies were initiated so that the fate of the extract emulsified in mineral oil might be determined. To our knowledge, radiopaque materials have not previously been used for this purpose.

TECHNIQUE

Of Visciodol®,* a uniform suspension of sulfanilamide in Lipiodol (40 per cent), 4.5 ml was used as the dispersed phase of an emulsion of which the continuous phase consisted of mannide mono-oleate (Arlacel A) 1 ml and 4.5 ml of mineral oil (Drakeol 6 VR).

By means of a Brown Emulsor, the three ingredients were repeatedly forced through an emulsator consisting of a double-hubbed hypodermic needle 5 cm in length and of an integral diameter of 0.63 mm at a pressure of 30-40 pounds. The comparable emulsion for use in humans is made by means of a similar emulsator excepting that the integral diameter is 0.305 mm.

The degree of emulsification was evaluated by microscopic comparison with erythrocytes (diameter 7.5 μ). By these and a number of other methods, the absence of free extract and the diameter of the emulsified droplets could both be determined. The coarsest emulsion in use contained droplets of which the diameters varied from 0.1 to 3.75 μ. At present, the droplets vary no more than 0.1 μ to 1.0 μ, with the larger droplets noted as one in ten to twenty fields.

For the preliminary experiments, five guinea pigs were used. Of the radiopaque emulsion, 0.1 ml was injected subcutaneously into the shaven abdominal area. A 150 KV Westinghouse X-ray unit was used with

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*E. Fougera & Company, Inc., Hicksville, Long Island, New York.

copper radiation (35 KV-15 milliamperes). The location was recorded on ultra-speed dental film with an exposure of three to ten seconds.**

The entire abdominal wall including the tissues surrounding the site

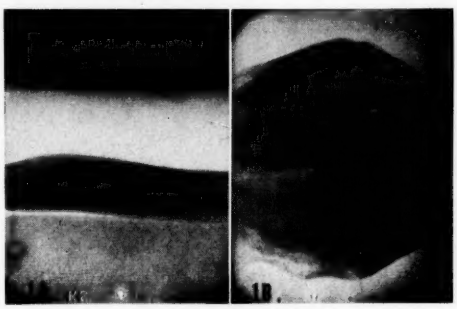

Fig. 1

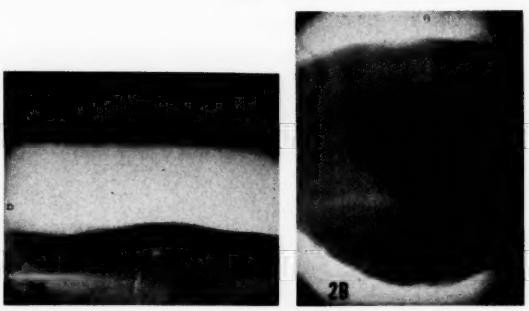

Fig. 2

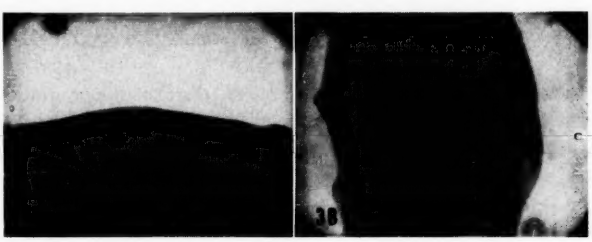

Fig. 3

of the injection was removed from the first test animal at the end of twenty-four hours and from each of the others on successive days. The horizontal and vertical aspects of each of the sites were roentgenographed.

The first figure illustrates the appearance of the site of the inoculation.

**The radiographs were made by Wayne M. Beasley, Engineering Experiment Station, University of New Hampshire, whose technical assistance is gratefully acknowledged.

As viewed from above, there is a limited lateral spread. This is equally apparent in the films labeled 2A and 2B which represent the roentgengrams taken at the end of forty-eight hours. The remaining roentgengrams show a considerable amount of the emulsion to be present at the site of inoculation as determined at the end of the fifth day.

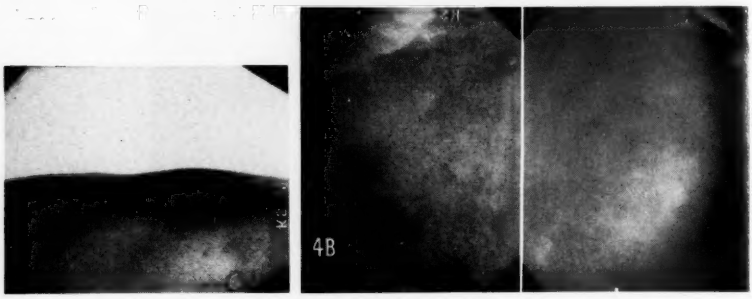

Fig. 4

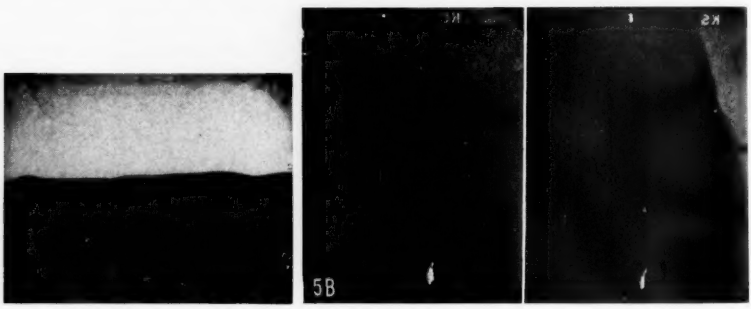

Fig. 5

DISCUSSION

The method makes it then possible to discern the minute, discrete globules of a mineral oil: emulsifying agent: extract type of emulsion deposited subcutaneously. It is also possible to follow the movements of the globules as they represent the degree and the rate of dissemination.

This study shows that some migration is noticeable and chiefly during the first forty-eight hours, but at the end of the fifth day, the greater part of the injected emulsion is obviously fixed in position.

A separate communication will concern itself with the subcutaneous inoculation of other types of emulsions into the limbs of test animals as quantitatively studied for longer periods of time.

REFERENCES

1. Brown, E. A.: The prevention of reactions to repository injection treatment. Ann Allergy, 15:499 (Sept-Oct) 1957.

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Basic Briefs

ALLERGIES OF THE GENITO-URINARY TRACT

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REVIEW OF LITERATURE

THE MAJORITY of the literature on allergy has concerned the skin and respiratory systems. Little involvement of the genito-urinary tract has been recorded. Duke[1] in 1922 wrote on food allergy as a cause of bladder pain. An excellent article in 1949 by Kindall and Nichols[2] reported on allergies of the pelvic urinary tract in the female. Eistenstaedt,[3] Unger[4] and Powell and Powell[5] have commented on allergic reaction causing symptoms of enuresis, renal colic, hematuria, hemoglobinuria, emotional irritability and albuminuria. Burkland,[6] Vaughan,[7] Rowe,[8] Kittridge and Johnson,[9] Goeltz[10] and others[11-17] have written pertinent articles on allergy of the genito-urinary tract. Powell and Powell[19] studied tissues from the bladder and urethra of 114 females on whom transurethral bladder neck resection had been done and found 5 per cent with acute allergic reactions.

Campbell[20] and Pelouze[14] stated they had never seen a case of proved allergic cystitis. Both admitted, however, that the relief of allergic cystitis by injections of ephedrine seemed to prove the existance of allergy.

Recently, several reports have compared the organic iodine dyes used for excretory urograms. Culp[21] found Diodrast gave the least serious side effects but was inferior in quality to other media. Myocon, Renographin and Hypaque gave superior urograms but had a higher incidence of allergic reactions. Nesbit[22] reported on a questionnaire study of nearly 150,000 dye injections, with 165 allergic reactions, 136 shock cases, and one death in the series. Pendegrass[23] in 1955 surveyed thirty-one deaths occurring in 3.8 million cases having intravenous pyelograms.

Lapides and Boyd[24] gave intravenous Benadryl prior to 70 per cent Urokon to 1500 patients, and only one exhibited urticaria and bronchospasm. Wechsler[25] and Nesbit[22] also believe that intravenous administration of an antihistamine prior to the intravenous pyelogram is of definite value.

Several observers[22,23,25,26] feel that the occasional systemic reactions of hives, a feeling of warmth, substernal oppression, and the rare shock and/or

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death that occur are allergic reactions to the high organic iodine content of the intravenous dye. These complications are controllable by immediate intravenous adrenalin and treatment of shock. By using an antihistamine intravenously prior to the organic iodine dye, these reactions should rarely occur.[22,24,25]

DEFINITION OF GENITO-URINARY ALLERGY

There are three types of allergy involving the genito-urinary system: (1) contact dermatitis, involving the penis and scrotum in the male; and the labia, vagina and perineum of the female, (2) allergies of the lower urinary tract (urethra and bladder in the female; and urethra, bladder and prostate in the male), and (3) allergies of the upper urinary tract (kidney and ureter).

Allergies are caused by: (1) inhalents, (2) contact, (3) drugs, (4) food, (5) bacteria, viruses, rickettsiae, et cetera. Contact with drugs or irritants can cause allergic reactions of the genitalia. It is possible that a secondary allergic reaction in the urinary tract may occur as the kidneys eliminate the inhalants in the urine. However, the greatest number of allergic reactions in the urinary tract seem to be caused by food and drugs.

SYMPTOMS AND FINDINGS

Edema, swelling, inflammation and itching, with or without pain, are the symptoms of an acute allergic involvement of the male or female genitalia. There is no fever, but tissue swelling in the male may be so great as to masquerade as urinary extravasation. In the chronic state the symptoms are itching, pain and swelling, and the severity depends on the amount of secondary infection present.

When the lower urinary tract is affected by an acute allergic reaction, the symptoms are frequency and urgency of urination, dysuria, nocturia and a dull suprapubic ache. The temperature is normal and there is no pyuria. There may be flank pain, gross blood may appear in the urine, and occasionally acute urinary retention occurs. In other words, these are the same symptoms that occur when an infection of the lower urinary tract develops. Unless the physician considers allergy in the differential diagnosis, he will treat the case as a common cystourethritis or prostatitis.

In chronic allergy of the lower urinary tract there may be minimal or severe symptoms. The usual complaints are aching in the pelvis, back and thighs, nocturia, dysuria, frequency and urgency of urination. Pyuria usually is present so that the added problem of drug therapy in an allergic individual makes diagnosis and therapy difficult.

Clinically, I have not recognized allergy of the upper urinary tract, other than as a part of the total urological allergic condition. I think only the pathologists can help us with this phase of the problem.

DIAGNOSIS OF GENITO-URINARY ALLERGY

A careful history is important in any medical problem. However, in allergic cases a good history is absolutely necessary for correct diagnosis, successful treatment, and prevention of future recurrences.

A patient who has acute hay fever, asthma, or hives, may have urological complaints as a manifestation of a generalized allergy. It also is worthwhile to evaluate urological symptoms on a possible allergic basis if the patient has a close relative who suffers from a known allergy.

The allergic hives or dermatitis of the male or female genitals may be obvious to both patient and doctor, but unless allergy is suspected the case will be puzzling and frustrating.

There are cystoscopic findings that are suggestive of lower urinary tract allergy. A pale, swollen mucosa of the urethra and bladder may have areas of bulbous edema, surrounded by a zone of hyperemia from which blood may be seen to ooze. The bladder has a reduced capacity and the urethra and bladder are hypersensitive. The urinalysis may be normal or there may be blood and/or pus present. A Wright's stained urinary sediment may show a large number of eosinophiles, and a differential white blood count may reveal an eosinophilia. However, x-ray studies are of no value at all, unfortunately.

A useful and important diagnostic procedure is an accurate "food-symptom diary"[27] kept for three weeks. A person who is having repeated urological flareup is most cooperative if the reason for the diary is explained thoroughly. If the individual is "hard to convince" this diary may have little value.

If a suspected food is omitted from the diet for five days, then eaten freely on the sixth day, bladder symptoms usually develop within one to twelve hours if the patient is allergic to that particular food. The height of the reaction will occur in about four to six hours after ingestion. This cycle of testing by omitting and adding a suspected food, if tried three times with similar positive results, is good clinical proof that a food allergy exists.[27,28]

A long acting antihistamine may offer some degree of protection when taken along with a suspected or known food allergen, and can be considered as a "screening" diagnostic aid.

The difficult and puzzling case should be referred to an allergist for diagnosis and treatment.

TREATMENT

Basically the treatment of acute allergy of the lower urinary tract is symptomatic. Aspirin, heat, codeine and forcing fluids are standard, time-honored and usually effective. The patient is advised to eat a bland diet, to force water, tea and even carbonated drinks, but not to drink or eat the tomato or citrus fruits. A fluid intake of 3 liters a day will help wash out an inflamed and/or infected urinary tract. Heat in the form of hot com-

presses, heating pad, Sitz bath, diathermy or microtherm aid in the relief of pain and inflammation are suggested. When infection is present, I usually prescribe four days of medication (either Gantrisin gr. 7.7 four times a day, or Furadantin 50 mg four times a day). For severe infection I prefer chloramphenicol or tetracyclin (250 mg every six hours for three days). After the acute phase has subsided, I secure excretory and cystoscopic x-rays and urines from bladder and both kidneys for culture and sensitivity tests. Local treatment consists of urethral sounding, bladder lavage with instillation of 5 per cent argyrol; and prostatic massage in males. Antihistamines seem to help many of these acute cases.

In the chronic stage, the allergic reaction usually is masked by a superimposed infection which obviously should be treated with appropriate drugs. In patients who have repeated acute exacerbation of bladder infections an allergic basis should be suspected and investigated.

Once a suspected food is proved to be a source of recurrent bladder allergy, that food must be eliminated from the diet for some time. Ultimately 95 per cent of patients can resume eating this food if there is an interval of at least five days before it is eaten again. In the remaining 5 per cent of patients, there seems to be a permanent food allergy problem.

Dietary monotony and vitamin deficiency can be troublesome, but specialized care by an allergist will help.

DISCUSSION

For several years I have become increasingly conscious of a cyclic increase in the number of bladder and/or prostatic flareups starting about Thanksgiving and continuing through New Year's. There is a slight increase also noted about Easter. At these times there is a sudden change of diet due to traditional holiday foods. Also, there seems to be a slight flurry of lower tract symptoms when strawberries, tomatoes, and summer fruits appear in the markets. Hay fever season invariably causes a number of quiescent urological cases to develop urinary tract symptoms.

It has been my experience that 85 per cent of lower tract allergies are caused by the following five foods: (1) citrus fruits, (2) tomatoes, (3) condiments, (4) chocolates and (5) nuts.

An astounding variety of foods causing allergic reactions have been found and proved clinically. My first patient to have a sensitivity to Houston water made the diagnosis herself. She demonstrated to me how distilled water, boiled Houston water, or less highly chlorinated water would not cause bladder symptoms. Since that time I have had one other such case.

A doctor's wife has proved an egg sensitivity to all of us, but of interest was her discovery that she could eat a twenty-minute hard boiled egg, and eggs used in breads and cakes. However, any less thoroughly cooked egg will cause a violent hemorrhagic cystitis within eight to ten hours.

One lady was able to drink beer or to eat eggs on separate occasions but these at the same meal would cause acute hemorrhagic cystitis.

CHART I. FACTORS INFLUENCING GENITO-URINARY ALLERGIES

I. General health	(A) good		
	(B) poor		
	(1) acute—chronic		
	(2) resp.—G.I.—skin		
	hay fever—asthma		
	intestinal "flu"		
	hives		
II. Emotions	(A) fear—anger—worry		
	(B) happiness		
III. Sleep	(chronic deficiency)		
IV. Endocrine system	(A) hypofunction		
	(B) hyperfunction		
V. Dietary habits	(A) G.I. absorption index	(1) high	
		(2) low	
	(B) overindulgence	(1) occasional	
		(2) constant	
	(C) minerals & vitamins		
	(D) weight	(1) obesity	
		(2) malnutrition	
	(E) food selection	(1) monotonous	
		(2) varied	
VI. Muscle tone	(exercise—fatigue)		
VII. Enzymes	(deficiancy—altered G.I. absorption index?)		
VIII. Geographic	(A) weather (Barometric pressure?)		
	(B) seasons		
	(C) humidity		
	(D) altitude (?)		
IX. Hydration	(A) deficiency		
	(B) excess		

A number of our patients cannot eat Mexican or Italian foods, barbecue sauces, onions, pickles and home prepared sausages without a subsequent lower urinary tract flareup. Food allergies produce an edema of the bladder and urethra that allows stagnation of urine. Sooner or later this becomes infected and is evident clinically as an acute cystitis in females, and prostatitis in males.

One case has been observed in which tomato was proved to be the cause of recurrent acute urinary retention in a man who previously had a prostatectomy. Nickey and Montgomery[29] found six cases of eosinophilic granulomatous prostatitis in the literature, and added the seventh. All of these patients had an allergic background. Dr. Siceluff of Springfield, Missouri, has sent me microscopic sections of his previously unreported case of eosinophilic granulomatous prostatitis.

In 1940, I did an incision and drainage on a young male with typical findings suggesting acute urinary extravasation. The final diagnosis proved to be acute allergic genital dermatitis caused by wearing a pair of shorts that were new, unlaundered and gaudy with color. Within a week, there were two other cases, but they were not operated on since a lesson was learned from the first case. Patch testing the skin of these three patients with a piece of the underwear caused considerable local edema and itching.

The loss of sleep, alcohol intake, emotions, weather, endocrine and enzyme factors all seem to have some effect on the allergic state. Their relative significance remains an enigma at the present.

We know too little of how the kidney secretes urine, conserves water, gets rid of bacteria from the blood stream, filters out toxic products of metabolism and eliminates excess chemicals and electrolytes. And yet the urinary tract is but a part of the "sewage disposal system" of the body.

There is relatively little known about the enzyme and chemical reactions that occur as food is digested and absorbed. What produces a sensitizing process to a certain food in a particular organ of the body in some people is understood even less.

More studies by investigators should clarify these normal and abnormal processes. In the future this knowledge may help solve the problems of diagnosis, etiology, treatment and prevention of all types of allergy.

SUMMARY

The scant urological literature on genito-urinary allergy is reviewed. The dyes used in intravenous pyelograms have been studied by many observers who believe that the prior administration of intravenous antihistamine protects against serious allergic reactions. Genito-urinary allergy is defined as to location and type. The symptoms and findings of acute and chronic regional allergy are enumerated. Various diagnostic procedures and tests are mentioned, and treatment is discussed in some detail.

It is stressed that a food-symptom diary and the omission and the addition of a suspected food will diagnose and prevent many food allergy problems. The five main offending foods that seem to cause most of the lower urinary tract reactions are listed. The histories of several patients are recorded who had unusual foods or contacts causing allergic symptoms. The various factors that may effect the development of genito-urinary allergy are listed, but no attempt is made to indicate their relative importance. An undiagnosed allergy of the urinary tract has a clinical course that is bizarre, puzzling and frustrating to a conscientious urologist. Confusion often is caused by the secondary infection which may be present, and the lack of a standardized diagnostic laboratory test for allergy. An effort has been made to show that genito-urinary allergy does occur and that it is usually misdiagnosed by clinicians (including urologists) but that it can be diagnosed, treated and prevented.

REFERENCES

1. Duke, W. W.: Food allergy as a cause for bladder pain. Ann Clin Med, 1:117, 1922.
2. Kindall, L. and Nichols, T. T.: Allergy of the pelvic urinary tract in the female: a preliminary report. J Urol, 61:222, 1949.
3. Eisenstaedt, J. E.: Allergy and drug hypersensitivity of the urinary tract. J Urol, 65:154, 1951.
4. Unger, D. L., Kubik, F. and Unger, L.: Urinary tract allergy. JAMA, 170:1308, 1959.
5. Powell, N. B. and Powell, E. B.: Transurethral bladder neck resection in the female. J Urol, 80:479, 1958.
6. Burkland, C. E.: Manifestations of hypersensitivity in the genito-urinary system. Urol Cutan Rev, 55:290, 1951.

7. Vaughan, W. T. and Hawke, E. K.: Angioneurotic edema with some unusual manifestations. J Allergy, 2:125, 1930-1931.
8. Rowe, A. H.: Elimination Diets and Patient's Allergies. Philadelphia: Lea and Febiger, 1944.
9. Kittridge, W. E. and Johnson, C.: Allergic hematuria due to milk. New Orleans Med Surg J, 101:419, 1948-1949.
10. Goeltz, F. A.: Allergy in relation to diseases of the urogenital tract. J Urol, 37:60, 1937.
11. Dees, S. C. and Simmons, E. C.: Allergy of the urinary tract. Ann Allergy, 9:714, 1951.
12. Walter, C. K.: Allergy as a cause of genito-urinary symptoms. Ann Allergy, 16:158, 1958.
13. Speer, F.: Food allergy in childhood. Arch Pediat, 75:363, 1958.
14. Pelouze, P. S.: Office Urology. p. 707. Philadelphia: W. B. Saunders Co., 1941.
15. Powell, N. B.: Urinary incontinence in children. Arch Pediat, 68:151, 1951.
16. Urbach, E. and Gottlieb, P. M.: Allergy. p. 849. New York: Grune and Stratton, 1946.
17. Tuft, L.: Clinical Allergy. pp. 589 and 608. Philadelphia: Lea and Febiger, 1949.
18. Vaughan, W. T. and Black, J. H.: Practice of Allergy (3rd ed.) p. 1086. St. Louis: C. V. Mosby Co., 1954.
19. Powell, N. B. and Powell, E. B.: Vesical allergy in females. Southern Med J, 47:841, 1954.
20. Campbell, M.: Urology, Vol. II. p 1530. Philadelphia and London: W. B. Saunders Co., 1954.
21. Culp, D. A., Van Epps, E. F. and Edwards, C. N.: Comparative studies of urographic media. J Urol, 78:493, 1957.
22. Nesbit, R. M.: The incidence of severe reactions from present-day urographic contrast materials. J Urol, 81:486, 1959.
23. Pendegrass, E. P., Hodes, P. J., Tondreau, R. L., Powell, C. C. and Burdick, E. D.: Further consideration of deaths and unfavorable sequelae following the administration of contrast media in urography in the United States. Amer J Roentgenol, 74:262, 1955.
24. Lapides, J. and Boyd, R. E.: The effect of intravenous benadryl in allaying allergic manifestations of 70 per cent Urokon. J Urol, 75:1016, 1956.
25. Wechsler, H.: Further studies of reactions due to intravenous urography. J Urol, 78:496, 1957.
26. Youngblood, V. H., Williams, J. O. and Tuggle, A.: Reactions due to intravenous Urokon. J Urol, 75:1011, 1956.
27. Rinkle, H. J., Randolph, T. G. and Zeller, M.: Food Allergy. Springfield: Charles C Thomas, 1951.
28. Rowe, A. H.: Elimination Diets and the Patient's Allergies. p. 129. Philadelphia: Lea and Febiger, 1944.
29. Nickey, W. M. and Montgomery, P. O'B.: Eosinophilic granulomatous prostatitis. J Urol, 75:730, 1956.

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CAUSES OF ERROR

Now there are four chief obstacles in grasping truth, which hinder every man, however learned, and scarcely allow any one to win a clear title to learning, namely, submission to faulty and unworthy authority, influence of custom, popular prejudice, and concealment of our own ignorance accompanied by an ostentatious display of our knowledge. Every man is entangled in these difficulties, every rank is beset. For people without distinction draw the same conclusion from three arguments, than which none could be worse, namely—for this the authority of our predecessors is adduced, this is the custom, this is the common belief; hence correct.—ROGER BACON, 1214-1294, *Opus Majus,* Translated by ROBERT BELLE BURKE, 1928 (From *Moments of Discovery in the Origins of Science,* GEORGE SCHWARTZ and PHILLIP BISHOP, Basic Books, New York, 1958.)

Historical Document
1916

HAY FEVER, ITS PREVENTION AND CURE

W. P. DUNBAR

YEAR AFTER YEAR I have consulted botanists as to the best method of getting pollen in large quantities. Various methods were suggested to me, among others that of spreading large cloths over the meadows. I also sucked up into bottles large quantities of air in the attempt to get pollen, and attempted many other things. None of these methods met with more than moderate success, until finally I hit upon the simple procedure of shaking blooming plants, for instance stocks of wheat, and catching the dust that was shaken out. I succeeded better later by taking the ears shortly before they began to bloom and putting the stocks in water in a warm place. In this way I was soon able to gather pollen in large quantities, and, more important still, to isolate the pollen grains of different plants, free from all contaminations, including micro-organisms.

After having obtained in this way the pollen of rye, wheat and ray-grass pollen (Lolium perenne), I could at once begin to attempt the settlement of important questions. A minimal amount of the plant dust when introduced into my conjunctival sac, or my nasal passage, caused in a very short time most pronounced hay-fever symptoms. The same experiment on my laboratory assistant, who did not have hay-fever, had no effect at all. Within a few days I extended the scope of my experiments so as to include two hay-fever patients, who happened to be working in our institution, as well as three other assistants who did not have hay-fever. The result was always the same. The hay-fever patients reacted just as I did. Those who had no hay-fever and served as controls were not at all affected by the introduction of the pollen. These experiments were repeated later on very many patients and people without hay-fever, invariably with the same results.

The next important point that suggested itself to me was to determine whether or not this toxin was active at other times of the year than during the hay-fever period. Formerly, it had been urged against the pollen theory that with the same pollen which had been active during the hay-fever period, no results could be achieved at other times of the year. Thus, for instance, Sticker was of the opinion that Woodward had proved that pollen was inactive except during the hay-fever period. He therefore was forced to come to the conclusion that for the production of an attack there was necessary the disposition on the part of the individual and the

W. P. Dunbar, as quoted in *Hay-Fever, its Prevention and Cure*, edited by W. C. Hollopeter, Funk & Wagnalls Company, New York and London, 1916.

season of the year. The nature of the action of the season of the year was explained by some authors as a sort of an "Unstimmung," a sort of spring revolution. This explanation appeared to me very doubtful, because of the fact that this process occurs in European patients in springtime; in most American patients, however, in the fall.

My experiments on this subject resulted as follows: Pollen which had been carefully dried soon after gathering was active later at any time of the year. Pollen, however, which I enclosed in bottles in its fresh condition underwent certain changes, characterized especially by the formation of a liquid.

Pollen which had been spoiled in this way proved later to be inactive. I might add here that these observations explained the occurrence of sporadic cases of hay-fever in the winter time. Pollen which has found its way into a dry room can remain active until the winter season—indeed, for many years, as I have shown. One blossom which has remained for eleven years in the herbarium retained an undiminished activity. Pollen which, on the other hand, falls to the ground in the open air, is destroyed by the first following rain. The fact that the pollen is carried down out of the air by the rain clearly explains further the remissions on certain days which had hitherto been so difficult to understand. By means of the isolated pollen I had then met those requirements in my attempts at an etiologic explanation which I myself have considered necessary. The suspected agent, free from all impurities, when applied to a hay-fever patient, must produce hay-fever invariably, regardless of the season of the year. The same agent applied to a normal person must have no effect. These requirements had, I say, been met by experiment.

The grass pollen is so small that a single one can not be seen with the naked eye, yet its structure and chemical composition are very complicated. Many pollen grains are covered with hair-like prickles. Adherents of the pollen theory formerly believed that hay-fever was due to these prickles. They asserted that hay-fever patients were extremely sensitive to the mechanical stimulus of the prickles, and that normal individuals were resistant to their action. It is true that some of the pollen, which we formerly looked upon as the cause of hay-fever, had an uneven, prickly surface. Some of the first adherents of the pollen theory *(sic)* that those pollen especially were active whose blossoms had an intense odor. The disease was accordingly at that time widely called rose-fever, linden-fever, and so forth, instead of hay-fever. I was able to show that those pollen which most often cause hay-fever have a smooth surface. This is true of all grass pollen, of which I have examined thirty-two varieties. These have also no odor. The blossoming of the rose and of linden occurs at the same time as that of the grasses. In 1902 I was able to completely overthrow this belief in the activity of the linden. It happened that in that year the blossoming of the linden was delayed from three to seven weeks in our vicinity, that of the grasses occurring at the regular time.

The season for hay-fever was probably over at the time that the blossoming of the linden was at its height, and hay-fever patients were able to enjoy the odor of the linden without any ill effects. I can well understand the tenacity with which hay-fever patients cling to the belief that the dust of the rose and linden causes their symptoms. I myself banned from my home every rose and other odorous blossom during the hay-fever period, and felt certain that my suffering was thereby diminished. The relief was not an imaginary one, but was due to the fact that at the same time I kept my windows carefully closed.

At about the time of the blossoming of the grasses, the pine (Pinus sylvestris) also begins to blossom and produces such a plentiful dust that thick clouds of it can at times be seen. This is called sulfur rain. I proved, in spite of the opposition of many hay-fever patients, that the dust of this blossom was also of no consequence.

By such experiments as these and many others I was able to prove that only certain specific pollen could cause hay-fever. This was in direct opposition to Blackley's theory. Other pollen, including those possessing sharp prickles, were absolutely without effect.

The theory as to emanations, odors, ethereal oils, and so forth, had still to be considered. On opening a vessel that contains much grass pollen one gets an odor much like that of honey, which proved to be without effect on hay-fever patients. The odor of the linden, as well as that of the harmless rose, was proved to be without effect. There was still to be considered the question as to the action of the ethereal oils. An extract of oily and waxy portions of the pollen, when applied to the conjunctiva and nasal mucous membranes in small amounts, caused a burning sensation. This was quite different, however, from the peculiar sensation experienced by hay-fever patients, which is so distinctive that nothing can stimulate *(sic)* it. These extracts had more effect on normal persons than they had on hay-fever patients. The amount of these substances with which we come in contact in our ordinary walks is so small that they can surely not be responsible for any of our unpleasant sensations.

Grass pollen is distinguished from that of other plants in a marked way by the small rods which it contains, which look just like bacteria. Patton, in 1877, had already called attention to these rods. He believed that after they left the pollen grains they possessed a movement of their own, and he drew the conclusion that they constituted the active principle of the pollen. He asserted that by reason of their inherent motility they found their way into the mucous membrane and the circulation and thus caused the symptoms of hay-fever. For a time, I also believed that these small rods played some part in the production of hay-fever. I did not know then that they were composed solely of starch, but thought they contained albumin. After I was able to get hold of great quantities of grass pollen I was able to isolate these rods by means of repeated

centrifugation and washing. I was then able to prove that they were absolutely innocuous to hay-fever patients.

As a result of certain observations, to which I shall refer later, I was soon forced to the conclusion that the active principle of the toxin of hay-fever is an albuminous substance. The alcoholic precipitate from a saline extract of a comparatively small number of pollen grains had an intense action on hay-fever patients, but none on normal individuals. After I obtained large quantities of pollen I began my experiments with the isolated albumin. Against this method of procedure it was claimed that I had not been working with a true hay-fever toxin, but with a denatured poison. For this claim there is no evidence; the critics failed completely to show any proof for the correctness of their assertions. From a purely scientific viewpoint, it is certainly better to work with the isolated toxin than the whole pollen grain or an extract from them such as I used at first, before I realized that the toxicity was an attribute of the albumin alone.

Dr. O. Kammann, who investigated this matter at my request, was able to prove that the albumin fraction contains the toxin and that the globulin fraction is entirely inactive.

Having determined that the albumin of the pollen is the specific cause of hay-fever, it was possible now to carry out my experiments along quantitative lines. It is possible to extract the albumin from the pollen by means of saline solutions of proper strength and then to precipitate it with alcohol or to obtain it by dialysis, and then dry it. In this condition it retains its activity for many years.

The experiments which I had done up to this time on hay-fever patients had not conformed to the natural process. In order to conform to these more closely, I performed the following experiment: A hay-fever patient and a normal individual took their places in a large glass cabinet in which rye pollen had been distributed. The hay-fever patient took sick, the other remained well. It was not determined by this experiment how much of the pollen had been taken up by the hay-fever patient. The question as to whether or not enough pollen was present in the air during the hay-fever season to cause the symptoms had not been satisfactorily settled. Blackley *(vide supra)* had already made attempts to settle this question by means of a method worked out by Phoebus. He had carefully counted at different periods of the years the pollen which gathered on glass plates, whose surface had been covered with glycerin. My co-workers, especially Liefmann, found that in the heart of Hamburg, while hay-fever was at its height, 250 pollen grains accumulated on a surface of one square centimeter during twenty-four hours, i.e., 25,000 to the square meter. It was established that with the first appearance of the pollen in the air the patient began to complain of an itching at the inner canthus of the eye; his suffering became more intense in direct proportion to the quantity of pollen in the air. On rainy days no pollen accumulated on the glass plates,

altho *(sic)* they were protected from the rain. Early in June the pollen of the grasses far exceeded those of other plants in numbers, and from about the third week in July grew gradually less, so that at the end of July, or the beginning of August, only a few stray grains were found. Thus can be explained the periodicity of the course of hay-fever and also the occurrence of sporadic cases after the hay-fever season is over. There was still no certain method of predicting quantitatively the action of the pollen. Dr. O. Kammann had shown that the organic portion of the pollen of the grasses is about 40 per cent albumin. He had shown further that about 20,000,000 rye pollen weighed one gram. By means of these figures we could compute the amount of toxin in a single pollen. By means of a solution of known strength of the poisonous albumin of the pollen, it could be determined how many pollen grains were necessary in any given case to produce mild, moderately severe, or severe symptoms. It was evident that different patients were susceptible in varying degrees. A concentrated solution introduced into the conjunctiva or nose of a normal individual causes no symptoms. The majority of hay-fever patients were stimulated by one drop (1-20 to 1-30 cm) of a solution of 1 to 20,000 or 1 to 30,000. There were patients, however, who responded to one drop of a solution diluted a million times, the equivalent of the amount of albumin contained in one to three pollen grains.

Liefmann constructed an aeroscope by means of which he was able to determine how much pollen was taken in with each breath. In the neighborhood of a field of rye one inhales with each breath two or three pollen grains; in the middle of a large city he found that in every cubic meter of air there were about three hundred and eight.

Thus in this way questions as to the quantitative relationship of the pollen to the attack were satisfactorily answered. By means of these experiments it had been plainly shown that the albumin of the pollen of certain plants, especially that of all grasses, is to be looked upon as the active cause of hay-fever. With my co-workers I examined the pollen of 106 plants, and found them all without any action, altho *(sic)* I had examined such pollen which had been considered capable of producing hay-fever. In addition to the pollen, I had been informed that in China at the time of the blossoming of Ligustrum vulgare a disease very much like hay-fever was prevalent. I examined the pollen of this plant and found it active. In Southwest Africa, when the grasses blossom, conditions like hay-fever prevail, especially among the half-breeds. One European had to forsake Africa at this time on account of his intense suffering. In Europe he remained perfectly well. On examination it was found that he did not react to the grass pollen. In Africa, however, at this time, the acacia blossom *(sic)* and it has been looked upon as the cause of the condition. This patient was unaffected by the pollen of two different species of acacia. I am in hopes that experiments which we have since then set on foot will explain this disease to us. In addition to the thirty

varieties of graminaceae and cyperaceae, I have found the pollen of the following plants active: swamp-pink (Lonicera caprifolium), lily-of-the-valley (Convallaria majalis), hairy Solomon's seal (Polygonatum multiforum), Oenothera biennis, rape (Brassica napus), and spinach (Spinacia oleracea).

Of special importance is the autumnal catarrh, which occurs in the United States of America, beginning early in September and lasting about six weeks. This autumnal catarrh is much more common in the United States than the vernal catarrh. I have had the opportunity of examining a large number of American hay-fever patients, and was able to establish the fact that those patients who only suffer in the fall do not react at all to the albumin of the grass pollen. They do react, however, regularly to the albumin of the pollen of the golden rod (Solidago) and of ragweed (Ambrosia). I have examined a large number of species of both these plants and they were all active. These patients also react to the pollen of the chrysanthemum and the other asters. Those American patients who suffer only from spring catarrh, not from the autumnal type, react only to the grass pollen, not to that of the golden-rod or the ragweed. A third group of patients suffer from about the middle of May until early in November with a hay-fever-like affection. These unfortunates react both to the grass pollen and to the active agent of the autumnal catarrh. Goldenrod and ragweed are very widespread in the United States. They are found not only on meadows, fields, roads and along edges of woods, but grow in the midst of cities in neglected places. In Europe they do not occur naturally, indeed goldenrod can with difficulty be made to blossom there. The pollen of goldenrod, however, is not scattered as easily as that of the ragweed. All attempts to grow ragweed in this country failed until 1911. In this year, which was extremely hot and dry, I succeeded for the first time. These facts serve as further important supports for the pollen theory. There were, however, still many questions to be settled before all this *(sic)* mysterious phenomena which characterize hay-fever could be explained.

One very important question, that of individual predisposition, I have only lightly touched upon. It is clear that all people, including the inhabitants of large cities, are at certain times of the year exposed to the pollen of many plants, which settle on their skin, conjunctiva, are inhaled into their nose, and taken up into the mouth. By far the largest part of these individuals are unaffected by the pollen, only a very small percentage take sick *(sic)*. The poisonous albumin of the pollen is a substance, therefore, which is innocuous to most people, and is only active in those cases which there is a special susceptibility. In other words, hay-fever requires an individual predisposition. This personal predisposition is present for the well-known poisons of the pharmacopoeia, either not at all or at most in very slight degree. In the case of the infectious diseases it is much more evident. If, for instance, the cholera or typhoid bacillus is spread

through a city by means of the water supply, only a small percentage of the inhabitants are unaffected. This can be explained on the theory that the cholera organism does not find in most individuals those conditions which are necessary to its existence and growth *(sic)*. The fact that only about half of the cholera patients die can be explained by similar quantitative differences. I do not know of another instance of a substance absolutely inactive as far as a part of the population is concerned, but a very virulent poison for others. The individual predisposition in a case of hay-fever must be of a peculiar sort. It might be explained that the hay-fever poison enters the circulation of some people (hay-fever patients) and not of others. That this is the case I could prove by the demonstration of antibodies in the blood of the hay-fever patients. I shall return to this subject later. Here it is sufficient to say that these specific substances could be found only at the close of the hay-fever period; six months later they had disappeared. In normal individuals, they could at no time be demonstrated. The gradual disappearance of the immune bodies is easily explained. We know from animal experiment that these substances appear a certain time after infection, gradually to disappear again. At first blush the demonstration of immune bodies in the blood of hay-fever patients would seem to be a sufficient explanation of the hay-fever predisposition. Close study makes this seem uncertain.

On continuing the experiments I found that these immune bodies were not present in all patients, indeed, in the same patient I could not find them in some cases two years in succession *(sic)*. The following objection to this explanation was even stronger: A colleague of mine, disposed to hay-fever, who had helped me for many years, allowed himself to be injected with a solution of pollen albumin. One half-hour after the injection marked symptoms appeared in the eyes, nose, and mouth. The patient experienced pains in the chest, expectorated a tenacious, mucoid sputum, and perspired freely. The respiration became rapid and difficult, the pulse-rate was accelerated, and the voice grew weak. After fifty minutes there was a flat, urticarial eruption over the whole body. After twenty-four hours all the results of the injection had not yet disappeared. At the site of the injection, there was a marked swelling which persisted for five days.

Injection of hay-fever toxin caused the same symptoms in me. A colleague who did not suffer with hay-fever reacted to the same dose with a small, almost imperceptible swelling at the site of the injection. Pollen albumin was, in other words, not toxic for him when introduced under the skin. Hundreds of experiments have proved to us that pollen albumin is not a poison in the ordinary sense of the word, and that even when introduced into the circulation it is inactive. Not only is the skin of hay-fever patients permeable *(sic)*, for the hay-fever poison in varying degrees, but it also reacts to the toxin in different ways in different patients. In some cases when a solution of pollen albumin is placed on the skin, there

occurs within a few minutes an erythema. If, on the other hand, a patient is very susceptible to hay-fever, the skin may show absolutely no reaction when brought in contact with the toxin. These results may be of value in the study of individual predisposition, since they enable us to throw some light upon the question as to whether or not hay-fever is to be looked upon as a result of a hypersensitiveness.

In the first place, statistics have definitely proved that hay-fever has no relation to any constitutional disease—for instance, gout; that, indeed, only a very small percentage of hay-fever patients are gouty. It is very commonly believed that hay-fever is due to some anomaly or stopping up of the upper air-passages. A local disease of the trigeminus is assumed by some, with a resulting sensitiveness on the part of certain mucous membranes. The falsity of these conceptions is clear from the experiments cited above. Not only does the whole skin-surface of many patients react to the poison, but subcutaneous infection is followed by characteristic effects. By showing that the anal mucous membrane of hay-fever patients reacts to the pollen toxin, I believe that all those hypotheses, which assume only a local sensitiveness on the part of the cranial nerves, or the capital mucous membrane is *(sic)* robbed of all support. Suggestion, as we have seen, plays a large part in the attempts to explain hay-fever predisposition. I can treat this question together with that of the role of specific odors of flowers, cats, dogs, etc. Two colorless, odorless solutions were prepared, and a drop from one of them placed on the mucous membrane of the eye and nose of a large number of hay-fever patients. Some reacted, others did not. None of them knew what sort of a solution was being used. The applications were then made in a different way, each patient receiving a drop of the solution which had not been used in his case before. Those who had reacted the first time did not do so the second. The one solution was physiologic salt, the other pollen albumin. None of the patients reacted to the saline, all reacted to the other solution. In the course of many years I repeated these experiments with many variations. In the place of the salt solution I used solution of albumin from inactive pollen. The results were always the same. In the face of such results, he who would explain hay-fever on the ground of suggestion *(sic)*, simply ignores all the facts to the contrary, and his opinion does not deserve serious consideration.

Hay-fever is looked upon as a result of an advanced culture and civilization. It happens that there are very few hay-fever patients in the laboring class, and that the Anglo-Saxon races, especially the Germans, English, and Americans, furnish the largest number of such patients. That hay-fever does at times occur among the Romans and other nations, I am able to gather from correspondence that I have had with inhabitants of such countries. In St. Louis, I met an elevator boy who had hay-fever. Among the Anglo-Saxons the disease is found most often among professional men. Men appear to be twice as susceptible as women. It is often claimed

that hay-fever follows a period of strenuous mental work, or of excitement, as, for instance, after examination, or in officers after maneuvres. Hay-fever has often been shown to be hereditary. Most frequently, however, a severe attack of influenza has left hay-fever in its wake. Other causes, as for instance, a difficult labor, are asserted by patients to have been the exciting cause of their hay-fever attacks. May we conclude from all these facts that hay-fever is the result of a disturbance of the central nervous system?

It was formerly believed that all hay-fever patients were nervous and exciteable *(sic)*. This is certainly not universally true. If we are indeed dealing with a severe abnormality of the central nervous system this, in most instances, makes itself felt only in a hay-fever predisposition. Hundreds of hay-fever patients have written me that except during the season they are altogether well, and I have found among hay-fever patients many with phlegmatic dispositions. Those idiosyncrasies, which resemble hay fever in a way, as, for instance, susceptibility to strawberries, crawfish, iodin, antipryin, bromids and the salts of quinin, are to-day explained on the ground of anaphylaxis.

Th. Albrecht declares that ten years ago every physician had his own theory concerning the treatment of hay-fever. And I may add from my own experience that every patient also had his own method of treatment, which was, as a rule, very complicated. From my records it is very evident that many patients had ten or more hay-fever remedies, which they used either separately or together. A hay-fever patient takes up at once every new remedy that appears and enthusiastically recommends it to others. As a rule, he learns of the new remedy near the end of the hay-fever season, and while he is using it his troubles disappear and he attributes his relief to the remedy he has been using. In the following spring he is undeceived. In this way, one hay-fever remedy after another is consigned to oblivion only to reappear later under a different name. The only remedies that have survived for any length of time are those with narcotic effects, such as cocain, adrenalin, anesthesin, morphin, etc. Concerning the danger connected with the use of these narcotics, it is surely not necessary to say a word. In addition, adrenalin and anesthesin and the remedies prepared from them cause in many cases a sensation that is much more annoying than hay-fever itself. I, myself, have tested all the hay-fever remedies on which I could lay my hands within the last ten years. With no one of them did I accomplish a beneficial result. There was indeed no reason to suppose that the remedy could accomplish the things that were claimed for it. It is easy to gather the same opinion if one reads the thousands or more *(sic)* hay-fever histories that I have in my possession. I have called attention above to the fact that on purely theoretical grounds nothing was to be expected from these preparations, and that chance had not put into our hands a chemical preparation that was effective. Every physician must warn his patients against the use of

narcotics. I shall, therefore, not consider those remedies and methods of treatment that belong to this category.

In the thousands or more *(sic)* histories which I have been able to read, cauterization, burning, chiseling, and sawing in the nose play a large part.

It has been shown that the active albumin pollen is a substance of such marked specificity that the albumin which causes hay-fever symptoms in one patient (pollen of the grasses in Europeans) is entirely inactive in other patients (those with autumnal catarrh). On the other hand, the toxin of the ragweed has absolutely no effect on the European patients. By means of the complement deviation method, I could prove that this specificity could be shown in the hemolytic properties of the different albumins, the albumin of the grass pollen reacting altogether differently from that of goldenrod and ragweed. In view of this state of affairs, it is not hoped that chance would furnish us a chemical substance that would either neutralize or render inactive the pollen albumin, or overcome the individual predisposition, which, as we have seen, is also strictly specific. I have come to the conclusion that we can accomplish our end in three ways only.

First, by finding localities where the specific cause does not occur; second, by protecting the eyes, nose and mouth of the patient from the pollen; third, by active immunization against the toxin, or the use of a specific antitoxin *(sic)*.

The first method is yearly employed by many patients with success. The second method is also successful. Hay-fever patients are free from symptoms in regions in which a specific pollen does not occur. This was to be expected from what we have learned about the cause of the disease. Thus many patients find relief on the seashore, in islands, and in barren mountainous districts. In Germany they go to Hellgoland. In the United States they retire to Fire Island, Long Beach, the White Mountains, Green Mountains, or Adirondack Mountains during the hay-fever season.

All my attempts of many years to get rid of the irritating contents of the horse serum have been in vain. As early as 1905, I realized that this would be so, for I proved then that the irritating substance (as I then called the anaphylactic agent) was bound to the euglobulin of the horse serum as was also the antitoxin itself. If the euglobulin is destroyed, the antitoxin is at the same time rendered useless. I have been able to help patients who have become anaphylactic in two ways: First, by the use of pollantin R., and the suggestion to use this diluted serum only before the occurrence of the hay-fever attack, in the very smallest doses, and if possible only once daily. Patients who follow these directions have informed me that pollantin R. did not irritate them at first, altho *(sic)* it did so later. The irritation was, however, not severe and disappeared within ten to thirty minutes. After this the patient was free from hay-fever attacks for one or more days. Secondly, I have taken advantage of the fact that horse serum anaphylaxis is in most cases specific, but does not appear to

me to be always so. I have seen instances in which during the development of a hypersensitiveness to one animal serum, patients were rendered anaphylactic to the sera of other animals also. This does not, however, happen often. I have, therefore, administered to patients who could no longer stand pollantin R. a very active rabbit serum with good results. It was not only possible to ward off attacks with this serum, but also to protect patients from further attacks during the hay-fever season. These results appear to me to support the opinion, which I exprest years ago, *vis.*, that the reaction to the antitoxin, and with it the tendency toward definite immunization, is directly proportional to the degree to which anaphylaxis toward animal serum develops. Patients who have become anaphylactic get along with much smaller doses of antitoxin than other patients, and have, I believe, a better chance to effectually overcome their hay-fever predisposition.

This is the goal toward which we must strive. I know of many hay-fever patients, some of whom had attacks of the worst kind, who were entirely free after the use of pollantin for a very short time. I consider these people permanently cured, and so expressed myself in an article last year. A rhinologist interested in hay-fever wrote to me that he could not understand these successes, and that he and his colleague had never been able to obtain such results. He wrote further that my experiences were in marked contrast to the experienced German Hay-Fever Association. In answer to such communications I have placed my material in the hands of the secretary of this Association, Dr. Th. Albrecht. I was very much pleased to learn as a result that Dr. Albrecht had been able to cure and successfully immunize patients by means of pollantin. In a recent publication, Dr. Albrecht reported twelve cases in which, after the use of pollantin, for a short time, there resulted either a complete cure or at least a marked improvement. These observations are, of course, of intense interest to me. I have only been strengthened in my former opinion that by means of a mixed passive and active immunization a permanent cure of hay-fever can be accomplished.

THE CREATIVE SCIENTIST

True creativity is as rare in science as it is in literature and the arts. It is revealed by the ability to crystallize meaningful concepts out of observations which at first glance are chaotic and patternless. The creative scientist searches for the comprehensibility and the underlying unity in the structure of the universe. He also recognizes that at any time these approaches to concepts of unity are but temporary, partial successes which must inevitably be replaced by new developments and new concepts.—From the Foreword to *Moments of Discovery in The Origins of Science* (Basic Books, New York, 1958) GEORGE SCHWARTZ and PHILLIP BISHOP.

Progress in Allergy

MICROBIAL ALLERGY

A Critical Review—1950-1960

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In view of the progress that has been made in recent years in the role that allergic reaction to various microbes plays in the production of disease, one cannot help wondering why more physicians have not been more receptive to its concepts and the application to their practice.

Even fifteen years before von Pirquet invented the word *allergy* (1906), Koch had demonstrated bacterial (tuberculin) hypersensitivity. Later work firmly established the existence of such a bacterial allergy and clarified to a considerable extent its role in tuberculosis.

In clinical practice, the delayed type of microbial response is infrequently encountered, possibly because it is seldom looked for. It has been noted in brucellosis, lymphogranuloma, histoplasmosis, and syphilis. The anaphylactic type of response to bacteria is still more infrequent. The typical wheal response will appear in about one in 500 patients routinely tested with diluted bacterial vaccines (Forman). Delayed readiness occurs more frequently. From the viewpoint of diagnosis, however, such positive tests are sometimes difficult to correlate with the presenting trouble, since the positive reaction may represent a past infection and may not be related to the present illness. It is helpful, of course, if a similar organism can be recovered from the infected tissues or their secretions. Positive identification can be made if the patient is thrown into an attack upon the injection of the vaccine. These facts have very decidedly limited the use and value of such tests. The other biologic methods proposed for testing for hypersensitivity are very time-consuming and expensive, and so have not received the attention that their worth demands.

It is apparent that the allergic response of the host to bacteria may manifest itself in one of these ways: (1) as an immediate response with wheal formation to full-blown anaphylactic response, or (2) the delayed indurated type which is usually referred to as "tuberculin late reaction." The persistent use of the word "allergy" to cover all types of clinical allergy, but with the atopic type uppermost in mind, has, in many instances, formed a mental block in the mind of the physician. Finally, the failure on the part of workers to decide to which of these three types of responses they refer when they speak of "bacterial allergy" has contributed more than anything else to the confusion that exists today about the allergic response of the human being to bacteria. As one would expect, a conflict arose between the unitarian concept of allergy and the desire on the part of some workers to keep the several of allergic response separate for the purposes of investigation and clinical diagnosis.

Today, there is much more uniformity of agreement about the part allergy plays in the course of an infection than the part that microbes and

the toxic products play in the production of the so-called allergic diseases "hay fever," asthma, eczema, and migraine headaches.

Many allergists are now beginning to admit the infective factor in many patients with allergic manifestations. There is also an increasing recognition of the role which allergy to microbes plays in the practice of clinical medicine—even greater than that seen in the literature of several years ago. Then, too, the newer knowledge which investigators are bringing to us about the part that enzymes have in immunity and allergy promises to elucidate some of the problems in this field.

In 1958, I spent a few weeks in Europe. In addition to mine, the several papers in microbial allergy which were presented at the Third International Congress of Allergology in Paris (October 19-26, 1958) were all very well received. The members from Czechoslovakia, Russia, East and West Germany, Denmark, Sweden, France, Spain, and Argentina all showed great interest in our work with the patient's leukocytes in the filtrates of specific strains of bacteria. Explaining my concept of microbial allergy took up most of my time during the Congress. Furthermore, in my visits to the laboratories and clinics of different European countries, I found that the immunologists and the allergists recognized bacterial allergy as an important section of this general subject. Not only were they conducting research on bacterial allergy, but they were also using their knowledge of it in their clinics.

IMMUNOLOGY

Although bacterial allergy is one of the most important, it is, unfortunately, one of the least explored aspects of medicine. As our knowledge increases, the list of diseases suspected of being bacterial allergies, or at least those in which allergy plays a role, is constantly growing. Not only are rheumatic fever and certain types of infectious asthma thought to be bacterial allergies, but today the trend is also to regard bacterial allergy as a major factor in such diseases as brucellosis, syphilis, bacterids, certain cases of urticaria, angioneurotic edema, vasomotor rhinitis, iritis, uveitis, and periarteritis nodosa, as well as acute glomerulonephritis.

Enough progress toward confirming such concepts has been made in immunologic studies that we now have a clearer understanding of the possible mechanism of these diseases. The strict unitarians, who say that the only difference between anaphylaxis allergy in man "lies in the route taken by the antigen and hence the rapidity with which it reaches the shock organs,"[1] have abandoned the antigen-antibody concept as the basic mechanism in anaphylaxis and allergy. Instead, they have now accepted the view that two independent reactions are involved, an antigen-antibody reaction and a sensitization process, with the imperfection of the first causing the second. They say that an antigen-antibody interreaction, which may take place in the body fluids within the cells themselves, defends the body from harm by invasion. If this mechanism fails, then the overpowering antigen induces the formation of a specific proteolytic, toxic enzyme system. This constitutes the primary mechanism of sensitization. A later invasion of the antigen then will initiate toxic proteolysis and liberate various proteoses. These, in turn, will activate normal nonspecific proteinases and thereby start a chaotic proteolysis of the host's own proteins. It is these various toxic substances, resulting from partial proteolysis, which are responsible then for the local and constitutional manifestations of anaphylaxis and allergy. Certainly this concept offers a plausible explanation for what one sees in the Blatt-Nantz test.

NEW BASIC CONCEPTS CONCERNING THE MECHANISM OF SENSITIZATION

Bela Schick[2] presented his view of the mechanism of allergy at the April, 1956, meeting of The American College of Allergists. He believes that two forms of allergy exist. One is physiologic and therefore beneficial, functioning mostly in the presence of exotoxic disease bacteria. Its mechanism enables the host to fight diseases caused by invading pathogenic microorganisms and leads to immunity. The other form of allergy develops following the invasion of a foreign protein, which, being an alien substance, must be eliminated. Its mechanism is similar to the first type, but it produces an anaphylactic hypersensitivity which is harmful and therefore pathologic. Such an allergic response does not lead to immunity.

Boyd,[3] on the other hand, holds a quite different view. He believes that allergy and immunity are not the same mechanism and concludes that:

> . . . the available evidence, with the possible exception of the (still hypothetical) role of antibodies of the reagin type in parasitic infections, provides, at present, no basis for regarding the hypersensitive reaction as part of the mechanism of resistance. On the contrary, in view of the damage to the tissues of the host, which it often causes, it must be regarded as a liability, and interpreted as another example of the miscarriage of the generally beneficial immune process.

Carpenter,[4] moreover, says:

> . . . at the moment it is impossible to say whether immunity and allergy are part and parcel of the same phenomenon. However, it would not be particularly surprising to find, eventually, that these various responses of the body to foreign substances represent merely different trials and errors in the slow process of evolution. Certainly they will continue to provide investigators with fundamental as well as practical problems for many years.

Doddi[5] comments that, despite the great differences that allergy and immunity show in their manifestations, they must come from a common source and be dependent on each other, at least to a certain extent.

Sevag[6] offers another concept, for in his view ". . . the genesis of all allergic manifestations is traced to a single process." Any foreign reactive substance entering the animal system, without being immediately destroyed, will alter certain cell proteins, making these native proteins antigenic and therefore causing the abnormal reactions associated with hypersensitization. According to this concept, the sum of the injuries inflicted upon various proteins in the cell are directly, chemically responsible for the abnormal or allergic manifestations which are the symptoms presented by the patient.

Sevag believes that antibody formation is secondary to the establishment of a more basic mechanism of hypersensitivity in which the degree of hypersensitivity, or alteration, and the quantity of abnormal reaction products which damage the host's metabolic stream are of primary importance. Antibody, reagin, and similar substances are therefore abnormal or potentially toxic products resulting from foreign agents acting on the host's metabolic system.

As the seats of the determinants of enzymatic and antigenic specifications, proteins dominate and regulate the entire function of cells and tissues. And since these proteins possess potentially active biocatalytic centers and many polar or reactive groups, they are in a constant dynamic state. From this viewpoint, the allergic phenomenon is another manifestation of an active general process.

Also according to this viewpoint, the phenomenon of the secondary immune response is intimately related to the hypersensitive mechanism—

as foreign proteins, combining with the host's enzyme system, synthesize altered globulins or antibodies.

The appearance of antibodies in measurable amounts requires a latent period, and Sevag points out that during this time the mechanism of hypensensitivity becomes established and outlasts any period of antibody response. This hypersensitive state remains as a potentially active mechanism. It responds readily to secondary antigenic stimuli, functions more efficiently, and does not require the longer latent preparatory period which preceded the primary response for antibody synthesis. Not only does it afford a defense against infectious agents upon re-exposure of the host, but it can also be called into play when restimulated by allergic agents. On the other hand, an accelerated inflammatory response arising because of previous contact with antigen is a common aftermath to infection in many organisms. The characteristic feature of both the primary and secondary responses to antigenic and other stimulations is their specificity; it seems that the affected cell systems have been specifically sensitized.

Because hypersensitivity persists in the absence of antigen or antibody synthesis, Sevag is of the opinion that there is a self-duplicating mechanism innate either to the reticuloendothelial system or to a pre-existing system from which the reticular cells originated. This belief tends to be confirmed by the studies of other investigators toward inducing morphologic changes associated with the origin of the hypersensitive state. Further proof that a hypersensitive state can be induced through antigenic stimulation without the participation of immune mechanisms is found in the demonstrations of bacterial allergy in agammaglobulinemic patients in the absence of the ability to synthesize normal or immune gamma globulins.

An antigen can only introduce a combining specific modification into a globulin molecule by acting as a highly specialized biocatalyst. The specificity of the enzyme substrate reaction and the antienzyme reaction constitutes a unity within a given center of a protein. Consequently, Sevag believes that failure or success in attempts at hyposensitization may depend upon whether or not the system involves competitive reactions. On the basis of these observations, then, he concludes that the hypersensitive mechanism may be regarded as an adaptive process to escape destruction, and is a biologic prerequisite for the maintenance and perpetuation of the living form under changing conditions.

In the concept of Rich,[7] however, hypersensitivity is not a step towards immunity. As proof of this belief, he gives experimental results in his laboratory and quotes the work of Raffael.[8] One of the various methods used to dissociate immunity from hypersensitivity was desensitization. Although the prevention of the hypersensitivity reaction in both types of hypersensitivity can avoid unnecessary damage to the body, there is still need for a safer and more efficient method of desensitization than the present one. The medical profession is well aware that asthma, hay fever, certain gastrointestinal disturbances, as well as many dermatologic conditions, may be due to hypersensitivity; and yet it is not sufficiently appreciated that other types of serious and "even life-threatening lesions" can be the result of hypersensitivity.

Rich[7a] separates disorders caused by hypersensitivity into two categories: (1) primary disease of hypersensitivity and (2) diseases that are accompanied by sensitization. Primary diseases of hypersensitivity are those in which the disease state "is the result of hypersensitivity to agents that are not, in themselves, intrinsically harmful on usual contact, and in which, had hypersensitivity not developed, no significant lesions or symptoms attribut-

able to the agent would have occurred." Diseases accompanied by sensitization are those "in which disease is produced by agents which are themselves injurious, but in which the disease-process becomes intensified by the sensitization that develops as a result of contact with the agent." Examples of such agents are micro-organisms, viruses, and animal parasites.

The role of tuberculin-type hypersensitivity, especially in chronic infections, is highly important, both because of its power to intensify tissue damage and destruction and because of the debilitation and systemic effects that a given amount of infecting agent is capable of producing.

MECHANISM OF ANTIBODY FORMATION AND SENSITIZATION

In discussing how antibody formation and sensitization is brought about, Carpenter[4] states that, "No hypothesis of antibody formation can be universally accepted until it is known whether the persistence of antigen within antibody-forming cells is a necessary prerequisite for antibody manufacture."

Most present-day hyptheses of antibody formation are based on the assumption that antibodies are produced by molecules coming in contact with other molecules or cells which have been modified by antigen molecules. The template theory of antibody formation, for example, continues to be widely accepted. Boyd,[3] in maintaining that Haurowitz's template theory[9,10] is "most in line with what we know about protein synthesis in general" points out that the protein synthesis seems to take place in or around the nucleus and in the small granular particles of the cytoplasm. These sites of protein synthesis are rich in deoxyribonucleic acid. According to Haurowitz's theory, the cell has a repeated functioning pattern which acts as a template and is responsible for the marked specificity of the synthesized protein that constitutes the antibody. Amino acids are first deposited upon the surface of the template, each acid being identical with the one beneath it, and then are formed into a polypeptide chain by enzymes of the cathepsin type. Thus, a process analogous to crystallization takes place. The resultant new molecule, not being tied to the polar forces of the nucleic acid, takes on its typical globular structure and is released into circulation. The template can also synthesize other molecules like itself, but these are modified in electronic and spatial configuration through the presence of antigen. When duplicated in a new molecule, this modification constitutes the "active patch" of the antibody molecule.

According to Haurowitz, the enzymes that link the absorbed amino acids to form a new polypeptide chain are nonspecific. The specificity of the synthesized proteins is determined by the specific shape and electrostatic field of the template surface, for, if this were not so, then no specific protein would result. The formed proteins and genes are cast off as a two-dimensional replica and then enfolded into a three-dimensional globular protein molecule. The template can then produce other replicas. The genes need not be enzymes, as they are nucleoproteins whose specificity is determined by their protein content.

Burnet,[11] on the contrary, objects to Haurowitz's views because of the difficulty of accounting for the storage of such templates in the rapidly changing population of the cells concerned:

> Where, for instance, do colonies of obviously newly formed immature plasma cells that Coon finds in the medullary cords of lymph nodes, receive their templates from? If templates can replicate, the answer is simple. If they cannot, some very complex ad hoc assumptions need to be made to account, particularly, for the difference between primary and secondary responses.

According to Cushing and Campbell,[12] the theory of Pauling[13] on the mechanism of antibody formation is still the most stimulating. It was

Pauling who suggested that the chemical nature of the polypeptide chains always remained the same, but that the foreign antigen influenced the way the terminal ends of the chains folded or coiled to assume the final configuration of the globulin molecule. When the final shape is reached, the interatomic forces preserve a stable configuration. "The attractiveness of Pauling's theory lies in its logical simplicity and the tangible concept it provides for antibody specificity and structure" (Cushing and Campbell). The anamnestic reaction which, for example, takes place in "booster" immunization inoculations remains unexplained by any of the template theories. Nevertheless, Cushing and Campbell hold that the argument against template theories (that antibody formation persists long after antigen has disappeared) is not necessarily valid because "analytical methods at present are not sensitive enough to detect small traces of antigen which could easily represent many millions of molecules." In support of their position, they call attention to studies showing that small amounts of "fragmented" antigens may remain in tissue for several months.

MECHANISM OF ANTIBODY FORMATION

Burnet, in his recent book "Enzyme, Antigen and Virus," reiterates and elaborates on his "self marker" concept.[11] Believing that antibodies can be formed without the presence of antigens, Burnet, with Fenner in 1949,[14] postulated that antigen produces an adaptive change in cellular catalysts. Certain intracellular enzymes (self markers) whose normal function is to dispose of damaged or aged body constituents, may gradually become adapted to acting on similar molecules of foreign substances (*i.e.*, an antigen). The first antigenic stimulus causes the enzyme to adapt to the most characteristic features of the antigen, thus becoming an antibody. Additional antigen increases the number of antibodies, and, as the lymph and blood pick up the antibodies, resistance to the specific microbes or poison increases. The antibodies cannot reproduce themselves, but since they have the induced specific complementary pattern, they can combine with the antigen. This new character is then passed on to the daughter cells which continue producing antibody without antigen. Such production goes on at a gradually diminished rate as the modified enzyme reverts back to its normal form. As the authors pointed out in their original monograph, one of the weaknesses of this hypothesis was the absence of direct experimental evidence. This, Burnet believes, has now been overcome. Now the chief weakness of the hypothesis, according to Burnet, is its "reticence about immunological paralysis in the sense used by Felton (1949)." However, Stark[15] in 1955 has re-examined this phenomena by using pneumococcal polysaccharides labeled with C-14 leads, and finds that judged by radioactivity, the material remains in undiminished amount in the tissues. It seems to be unsusceptible to the normal processes of metabolism. If, however, the antigenicity of tissue extracts is examined by inoculating them into other mice, a progressive fall is noted. This is believed to be due to the antigen deposit being gradually blocked with adsorbed antibody. "In favor of this view is the fact that a mouse immunized by a small dose of polysaccharides will lose its immunity if subsequently inoculated with a large (paralytic) dose." Burnet also believes that:

> The type of antibody produced as the result of secondary processes, varies according to circumstances, even with the same antigen. It is probable, in fact, that by choosing appropriate conditions, a single antigen could be used to induce production of either (1) classical circulating antibody, (2) hayfever antibody, (3) tuberculin-type sensitization, or (4) specific immunological tolerance. It is a task of

the future to define the appropriate conditions more closely, but it is not unreasonable to believe that specific tolerance (above 4) normally characteristic of the prenatal reaction, be proved inducible also in cells exposed to concentrations of antigens which are beyond their ability to handle.

It is an established fact that many chemicals which, when applied epidermally or intradermally, produce a delayed type of reaction, cause an immediate type of reaction when injected parenterally. When certain adjuvants, for example Freund's adjuvant, are added to the injection, the intensity and frequency of the epidermal sensitization is greatly enhanced. This is the case when mycobacteria are used as an adjuvant in sensitizing guinea pigs to picryl chloride, egg albumin, or tuberculin. Here, the reactions become more numerous, more intense, and are of the delayed type. This can be explained, in Mayer's opinion[16-18] by the template theory. "It may be expected that an antibody formed on a template modified by an antigen containing globular proteins as the carrier will be different from an antibody molded on templates modified by the presence of complete antigens containing fibroid proteins as the carrier." Haptens, alone, introduced into the peritoneal cavity, generally produce an immediate type of allergic reaction, but when collagen in tubercle sessile antibodies is present, humoral antibodies and a delayed reaction are produced.

In developing this hypothesis, Mayer injected guinea pigs parenterally with picryl chloride using procollagen (a purified collagen) as the adjuvant. After the necessary sensitization period, the guinea pigs, upon epidermal challenge, showed delayed reactions in increased number and strength. Picryl chloride and procollagen produced the same "delayed-reaction-promoting effect" as tubercle bacilli or lipo-polysaccharides from the wax fraction of mycobacteria which form specific tubercles. These form fibroid protein, especially collagen, near the sites of the injected hapten, while the hapten combines with the collagen to form a complete antigen having a carrier of fibroid protein. These results are compatible with the theory that sessile antibody of the delayed type of sensitization is formed by complete antigen with fibroid proteins as the carrier. Humoral antibodies of the immediate type are formed by complete antigen with globular proteins as carriers.

In an editorial article, Campbell[19] considers the various theories of antibody formation discussed previously, and calls attention to the fact that one of the chief objections to the template theory is its assumption that antibody continues to form long after the antigen has disappeared from the tissues. As pointed out above, this objection stimulated Burnet and Fenner to propose that the antibody-forming mechanism depends upon an alteration of intracellular enzymes by the antigen. This they assume to be the same mechanism that operates in the formation of inductive enzymes. The antigen would, therefore, no longer be necessary once it had made its specific imprint on the enzyme system. In summarizing some of the investigations (providing strong evidence for antigen persistence) that have been carried out by himself and others, Campbell states:

These ideas lend strong support for theories of antibody formation which postulate the presence of antigen for each molecule of antibody that is formed. They attempt to correlate some of the recent information of protein synthesis and enzyme reactions with fragmentation of antigen and antibody formation.

THE PROBLEM OF DETECTING ANTIBODIES

At the recent International Symposium on mechanisms of hypersensitivity, Stephen V. Boyden,[20] Denmark, presented a paper on "the approaches to

the problem of detecting antibodies." The following comment is quoted verbatim:

> Although we know that there are important differences between circulating antibodies of the classical type and those which we are trying to detect for hypersensitivity, we nevertheless tend to take the classical antibody-antigen reactions as a model for our thinking of interaction between antibody and antigen in hypersensitivity. Even in the case of hypersensitivity of the tuberculin type where there is no shred of convincing evidence for anything like a globulin antibody being responsible for the reaction, we still tend to be looking for something in the tissues which will "combine" with antigen much as circulating antibodies can be shown to combine with tuberculoprotein. It is certainly difficult to imagine any kind of reaction as specific as the tuberculin test not being associated with the presence of some host factors with a specific affinity for antigen. But the interaction between these host factors and the antigen may be very different from the classical antibody-antigen reactions. It might involve, for example, only a very transient relationship, which our methods of measuring "combining capacity" would miss completely.

Boyden further suggests that the tuberculin type of sensitivity may be due, not to the presence, but rather to the absence, of certain factors. Perhaps many bacterial proteins are potentially toxic for cells, but under normal conditions the cells contain naturally occurring protective factors with some degree of specificity. Infection with certain bacteria might result, for various reasons, in the appearance of cells devoid of these more or less specific protective factors. We could imagine that the sensitivity of tuberculin is a reflection of the number of cells present which are devoid of the factors protective against tuberculoprotein. The polysaccharides might not be potentially toxic for cells so that delayed-type sensitivity to them cannot exist. Boyden concludes that "We can, however, receive some cheer from the fact that, although this view cannot be completely discarded at present, there are a number of recent experimental reports which, at least at first sight, tend to speak against it."

SITES OF ANTIBODY FORMATION

Since we do not yet know the sites of actual antibody formation, we cannot tell whether the cells manufacturing humoral antibodies are the same as those causing bacterial hypersensitivity. Early investigators felt that the macrophage cells were responsible for forming antibodies since they take up foreign material. This concept was reinforced by the discovery that a "blockade" of these cells results in the suppression of antibody formation, and that macrophages observed while living in tissue cultures appeared to manufacture globulin as well as to shed cytoplasm. Now, however, it is generally believed that a "blockade" cuts down the capture and production of antigen rather than the manufacture of antibodies. Nevertheless, as McMaster[21] points out, "the newer work has not yet shown that reticuloendothelial cells either do not or cannot form antibody."

In the past few years attention has been focused on the possible role of lymphocytes and plasma cells in the process of antibody synthesis of the delayed type of hypersensitivity.[22-25] Small lymphocytes, teased out under fluid from nodes of vaccinated animals, 70 per cent to 80 per cent viable, can transfer the power to develop antibodies to an irradiated recipient. Raffel,[26] however, does not regard the lymphocyte as the ultimate cell concerned in antibody formation because: (1) direct examination of tissues of vaccinated animals by tracer techniques shows the antibody residing in plasma cells;[27] (2) patients with agammaglobulinemia, and unable to form antibodies, lack plasma cells even in the lymph nodes regional to sites of vaccine injection;[28] (3) when total body x-ray irradiation is applied before

an antigenic stimulation, antibodies are suppressed and a latent period of several hours exists during which antigenic stimulation can still produce an almost normal response.[29,30] Raffel's interpretation of these studies views both plasma cells and lymphocytes as in some way involved in antibody production, probably during the early stage of reaction between antigen and the host's tissues.

Harris and Harris[31] have very comprehensively reviewed the present status of our knowledge of the mechanisms and sites of the synthesis of antibodies. Despite the vast amount of research of the past few years, it is as yet, according to them, impossible to determine what particular cell or cell types are involved or "whether the same or different cells are involved in the respective organs and under various conditions of antigenic stimulation." Sufficient evidence exists, however, for the participation of plasma cells and lymphocytes under various conditions. However, they maintain that in "any attempt to formulate a theory providing for the participation by both cell types in the synthesis of antibody, one would face several logical difficulties." First, disagreements exist among cytologists and others as to the characterization and interrelations of the cells themselves. Second, uncertainties exist regarding to what extent experimental conditions affect the cellular source of an antibody. Furthermore, we do not yet know the significance of such differences as those between single and multiple injections of antigens, grades of severity of antigenic stimulus, forms of antigenic material, as well as sites of injections and their relationships to the tissues studied. According to Harris and Harris, however, certain experimental data strongly favor the plasma cell or the lymphocyte. Their evidence in support of the plasma cells as the responsive cell to antigen includes:

1. The findings of Bjørneboe and his co-workers[32] that in rabbits, immunized intensively with pneumococcus, extracts of the fat of the renal pelvis containing cellular infiltrations contained more antibody than extracts of the other tissues not so infiltrated. There were 90 per cent plasma cells in these infiltrations and 10 per cent lymphocytes.
2. The observations by Fagraeus[33] that antibody was produced *in vitro* by fragments of red pulp, but not of white pulp, obtained from spleens of animals given secondary injections of bacteria.
3. The findings of Coons and his associates[34] that fluorescent-stained antibody was associated with groups of plasma cells in lymph nodes and spleen following secondary injections of antigen.

Harris and Harris also assembled typical experimental data to support the role of the lymphocyte in antibody production:

1. The finding of higher antibody titer in the cell sediment than in the lymph plasma of lymph draining from lymph nodes in the region of single injections of antigen. Ninety-nine per cent of the cells in the sediment of these experiments were lymphocytes.
2. The finding by Wésslen[35] of the production of antibody *in vitro* by cells taken from the lymph of the thoracic duct of animals injected twice with bacterial antigens. There were no cells in these suspensions other than lymphocytes, and no change of cell types was observed during incubation.
3. The preponderance of lymphocytes used in suspensions in recent cell transfer studies.[22,36] In these investigations in which the results of differential cell counts were reported, there were more than 95 per cent lymphocytes on the average in two studies, and in three others, 85 per cent, 95 per cent, and 99 per cent, respectively.

Thus, the conclusion of Wilson and Miles[37] in the new edition of their textbook is that:

The evidence strongly indicates the plasma cell or its immediate precursors as one of the sites of synthesis, but near relatives of the lymphocytes of the macrophages may also be concerned. At present we must be content with the generalization that antibody formation, like the uptake of antigens—whether inert or of living organisms—is part of the adaptive response of the multipotent cells of the mesenchymatous lymphoid-macrophages system.

Chase[22] is of the opinion "that we are handling, albeit in a mixed system, an enzymatic mechanism that functions in the formation of antibody," and the work of some investigators points to a protein antigen residing only for a short time in the lymphocytes.

Boyd,[3] in citing McMaster and Fagraeus[21,38] for support, believes that "The weight of evidence at the present suggests that the plasma cell rather than a lymphocyte, is the main source of antibody production."

Still another concept is held by Favour[39,40] who maintains that the lymphoid cell is responsible for the delayed type of allergy. He points out that "the one cell type which has become common to the various cell populations used for successful passive transfers, has been the lymphocyte." Another argument supporting such a view, according to Favour, comes from studies done on hypogammaglobulinemia in man. Humans with hypogammaglobulinemia have normal lymphoid cells in the blood and lymph nodes, as well as the ability to develop delayed type tuberculin reactions after BCG inoculation. Since no analogous condition is found in the experimental animal, Favour feels that this is a "unique example of a marked deficit in the gammaglobulin-plasma cell system, but a relatively intact lymphoid cell-delayed-type-reaction system." Furthermore, the delayed-type reaction can be passively transferred to hypogammaglobulinemic patients, who can be donors for peripheral blood leukocytes used to passively transfer delayed reactivity to normal subjects. Also in support of his standpoint, Favour reminds us that "in experimental animals, various tissue brews and washed neutrophile suspensions from sensitive donors do not passively transfer delayed-type tuberculin reactivity in normal subjects."

Antibody production according to Burnet,[11] on the other hand, is "a specialized function of mesenchymal cells and not something that is common to any type of vertibrate cells." In his opinion:

. . . the antibody-producing mechanism is initiated by the entry of the antigen into phagocytic cells of the reticulo-endothelial system (macrophages)—antibody producing units are transferred to reticulum cells or other relatively undifferentiated mesenchymal cells in the immediate vicinity of the macrophages. Under conditions inducing active antibody formation, these cells multiply freely and take on the staining qualities of plasma cells. They are responsible for the actual production of antibody during the peak phase of the response. The reticulum cells also give rise to lymphocytes, which fact is probably responsible for the maintenance of low levels of antibodies long after the antigenic stimulus.

Burnet believes that much of the recent experimental work supports this premise. For example: (1) The studies of Taliaferro and his associates on the effect of x-rays on hemolysin production in rabbits;[29,30,41] (2) the histological studies of Mauerer and associates,[42] who found almost no small lymphocytes at the height of the reaction; (3) the work of Harris' group;[43,44] (4) the finding of Coon's group that fluorescent-stained antibody was associated with groups of plasma cells and lymph nodes and spleen following secondary injection of antigen.

In addition to Burnet, Carpenter[45] believes that "both reticuloendothelial and lymphoid cells participate in antibody formation. The particular tissues concerned probably differ according to the site and route of inoculation."

P. G. Gell,[46] of the University of Birmingham, reviewed the nature of hypersensitivity tissue reaction during a symposium on the physiopathology of the reticuloendothelial systems held in Paris under the auspices of the Council for International Organizations of Medical Sciences. His summary of the origin and function of plasma cells is fully consistent with the theory of Daugherty:

> . . . the synthesis of antibody takes place during the differentiation of lymphocytes from fixed or free reticuloendothelial cells. According to this theory antigen incorporated into the phagocytic reticuloendothelial cell, a cell in which it is now known, may remain for a long period, and this initiates the synthesis of homologous antibody . . . the synthesis of antibody then proceeds in the various lymphocytes derived from the reticuloendothelial cells during the heteroplastic lymphopoiesis, and is probably complete in the mature lymphocyte. This theory could explain why reticuloendothelial cells contain little antibody and much antigen.

In the same volume, the article by Charles A. Doan[47] gives a more complete discussion of the role of reticuloendothelial cells in the disease. And for a good concise review of the various theories regarding the lymph cells involved in antibody formation, there is the recent book by Yoffey and Courtice, "Lymphatics, Lymph and Lymphoid Tissue."[48]

Some clarification on the site of antibody production may be expected from the work now being done with labeled antigens and with fluorescin antibodies as indicators of the localization of the antigen in the tissues. Also, we may expect still more promising results from the radioactive substances used in autoradiography. Although Coons in 1953[49] demonstrated the presence of injected antigen in phagocytic cells, no similar demonstration has yet been done with the polymorphonuclear leukocytes. Also in 1953, Dixon and his co-workers[50] showed that they are readily catabolized in the host.

But all in all, the literature on the sites of antibody formation is largely chaotic. Each school of hematology has its proponents for its particular cell —macrophages, reticuloendothelial, lymphoid, lymphocytes, or plasma cells. as the case may be. For the moment, then, we must agree with Haurowitz[51] who supposes that "since all cells produce proteins," all of them are also able to form antibodies, "provided they can trap the antigen and store it without destroying it."

ON THE ORIGIN OF PLASMA CELLS AND THE PRODUCTION OF ANTIBODY

Despite the great deal of literature on plasma cells, their origin and role in the production of antibodies remains a controversial subject. It is an established fact that repeated injections of antigenic material produce an increase in the number of eosinophils and plasma cells. Following a challenging injection, there is a progressive increase in the number of eosinophils at the injection site and in the regional lymph nodes until the fourth day. They then gradually decrease after six days, but with their disappearance, plasma cells appear and antibodies can be detected in the serum. If tritiated thymidine is used to label dividing cells, there is strong indication that the plasma cells are formed as the result of morphologic differentiation and not because the cell divides. Moreover, eosinophils are phagocytized by reticuloendothelial cells showing morphological changes, which then lead to an increased cytoplasmic basophilia. Because of these observations, Spiers[52] proposes a theory of antibody formation which takes into consideration the reaction of eosinophils with antigen, forming an enzymatic template. Phagocytosis of such sensitized eosinophils by reticuloendothelial cells causes the transfer of the template to the macrophages with subsequent antibody

formation taking place at the same time as transformation of the phagocytic cells into plasma cells.

In studying the function and life cycle of lymphocytes, Keohane and Metcalf[53] employed some physical techniques for determining the identity of cells isolated from various sources. They could determine the average cytoplasmic refractive index of small lymphocytes fairly accurately, and it was noted that chronically infected rats suffering from rat pneumonia had "a significantly raised cytoplasmic refractive index" of their small lymphocytes, regardless of the source. A similar rise, produced with subcutaneous injection of T.A.B., paralleled the rise in antibody titer of the serum and thoracic duct. These investigators believe that "the refractive index change in the cytoplasma of the small lymphocyte is due to increased protein concentration with the mechanism of production of antibodies." Further studies are presently being carried out.

ANTIBODY FORMATION IN TISSUE CULTURE

Despite the fact that antibodies produced by explanted tissue cells from immunized animals are demonstrable, most attempts to initiate antibody formation *in vitro* have so far been unsuccessful. Fishman[54] in a recent article described the *in vitro* initiation and production of two different antibodies in cultivated lymph node cells of normal rats. One of these antibodies was reactive with the bacteriophage T2, while the other was reactive with hemocyanin (busycon canaliculum). Further investigations are being carried out by Fishman to characterize the T2 phage-neutralizing substance.

DELAYED HYPERSENSITIVITY AND ITS POSSIBLE RELATION TO ANTIBODY FORMATION

Most, and perhaps all, living cells, as an adaptive mechanism, can form proteins of configuration complementary to inducer molecules. "Looked upon in a broad sense, the comparison between induced bacterial enzyme synthesis and antibody formation in animal cells is probably a meaningful one."[55] In both cases, we are dealing with a new synthesis of proteins having a structure complementary to that of the inducer, regardless of whether we are dealing with substrate or haptenic groups.

In discussing the delayed-type hypersensitivity reactions and their possible relation to antibody formation, Pappenheimer, Jr.,[55] and his group provide highly suggestive evidence that injection of antigen into an animal induces synthesis of two types of structures with configurations complementary to determinant groupings on the antigen molecule. One of these antigen-binding sites is fixed to cells and determines the phenomena associated with the delayed hypersensitive state. The other is the antibody gamma-globulin molecule itself. The synthesis of each of these two structures is under separate genetic control, while (for both) antigen is probably necessary as a template for directing the folding of polypeptide chains to complementary figurations.

Pappenheimer, Jr., and his colleagues believe that these two antigen-induced complementary structures are related to each other, inasmuch as they "represent successive steps in antibody syntheses. The primary antigen-induced complementary configuration is at or near the cell surface. It is the specific factor responsible for the inflammatory reactions characteristic of the delayed hypersensitive state. Secondary stimulation of sensitized animals with small doses of antigen may produce a delayed progressive inflammatory reaction. Larger doses of antigen may result in desensitization. But in either case, there is a specific interaction of antigen with the com-

plementary binding sites of certain sensitized cells. The antigen is then captured by the cell and retained at the site of gamma-globulin synthesis. Postulating that enzymatic action similar to bacterial permeases takes part in the transporting of antibody gamma-globulin synthesis is unnecessary. Such 'preplasma' cells produce antibody gamma-globulin and differentiate into plasma cells." A clonal distribution of antibody-forming plasma cells will result from secondary antigenic stimulation if, on proliferation of sensitized cells, the antigen-induced binding sites are divided so that the daughter cells remain hypersensitive (analogous to the progeny of preinduced bacteria).

Without the need to assume that each antigen can cause something similar to a specific directed mutation, this hypothesis explains the difference of primary and secondary responses to antigenic stimulation. Neither is it any longer necessary to assume that the antigen "selects" its own complementary configuration from a vast number of pre-existing "errors" in normal gamma-globulin synthesis.

STUDIES ON ANTIBODY PRODUCTION BY PERITONEAL EXUDATE CELLS

By steeping exudate cells from normal rabbits in heat-killed *Salmonella typhi* suspension for one hour *in vitro,* Fujino[56] cultivated the cells by means of a tissue culture method. The agglutinin production of these cultured cells was then studied and compared with the cultured spleen under similar conditions. Cells steeped in 0.01 mg/cc antigen suspension at room temperature had a weakened migration in the culture and their antibody production was not pronounced; while the cells steeped in 0.0005 mg/cc antigen suspension at room temperature migrated vigorously during the culture but did not have a remarkable antibody synthesis. Moreover, when they were steeped in 0.002 mg/cc antigen suspension at room temperature, marked migration took place during the culture and antibody was definitely produced. Cells steeped in 0.002 mg/cc antigen suspension in the refrigerator synthesized more agglutinins than at room temperature in the culture. Although the peritoneal exudate cells were only slightly inferior to spleenic tissues as far as their ability to produce antibody *in vitro* was concerned, they produced less agglutinin than the same cells from sensitized animals. The amount of antibodies synthesized usually rose on the second day of cultivation, reaching a maximum agglutinin production from the second to fourth day of cultivation.

Fujino[57] also investigated the anamnestic response of cultured peritoneal exudate cells after being steeped in the same antigen as in the previous *in vitro* treatment. The resulting agglutinin titers were lower than those produced by the same cells from sensitized animals in the first part of his experiments, and somewhat higher than those produced by cells steeped in antigen suspension *in vitro.* In this case, the antibody synthesis generally rose on the first day of cultivation, while the maximum agglutinin production took place earlier than previously. The peritoneal exudate cells, in this experiment, synthesized as much agglutinin as the spleen. Fujino believes that these findings show that peritoneal exudate cells steeped in antigen *in vitro* can produce bacterial agglutinin.

Fujino also made other studies with peritoneal exudate cells and reached the conclusion that it is still questionable whether or not agglutinin response in recipients is dependent on the antibody-producing ability of transferred exudate cells alone. But, he concludes, it cannot be denied that these transferred cells can synthesize bacterial agglutinin in recipient animals to a certain degree.

PURIFICATION OF ANTIBODIES

The obvious need of finding better methods for the isolation of purified antibodies is causing considerable attention to be focused on the problem. Oscar Swineford, Jr.,[58] and his co-workers recently published a series of experiments which they believe is the first demonstration of the successful purification of precipitated antibodies by enzymatic elution of the precipitating antigen. They found not only that dextran precipitated sensitizing and precipitating antibodies from Type II antipneumococcal rabbit serum, but also that all detectable anti-dextran precipitans were precipitated by dextran in the zone of hapten excess. Dextran produced anaphylactic shock in guinea pigs previously sensitized passively with Type II antipneumococcal rabbit serum, while dextranase denatured dextran in saline, in protein solutions, and in insoluble complexes with precipitated antibodies. Dextranase eluded all readily detectable dextran from dextran-anti-dextran precipitates. The resulting "purified antibodies precipitated with and passively sensitized guinea pigs against dextran. Antibody that was purified three times still sensitized against and precipitated with dextran. Purified antibody solutions still exhibited the phenomenon of dominant and recessive haptens."

Recently, these investigators reported two additional experiments, illustrating the phenomena of dominant and recessive precipitating and desensitizing haptens.[59] Type II pneumococcal polysaccharide precipitated all of the anti-Type II and antidextran precipitins from Type II antipneumococcal rabbit serum. Dextran precipitated all of the antidextran but not the anti-Type II precipitins.

They also found that Type II polysaccharide desensitized passively sensitized guinea pigs against dextran but not against the polysaccharide. In both of these experiments, polysaccharide was the dominant and dextran the recessive hapten.

MORPHOLOGIC ASPECTS OF ANTIBODY SYNTHESIS

Dixon[60,61] did cell transfer studies in order to determine which cells are capable of antibody synthesis and how much antibody a given number of cells can produce. First, lymph node cells and oil-induced peritoneal exudate cells from previously immunized donor rabbits were transferred subcutaneously to x-radiated recipient rabbits, and then the morphologic sequence of cell types in the transfer sites was observed during antibody response. Lymph node cells (90 per cent lymphocytes) as well as peritoneal exudate cells (70 per cent macrophages) can elicit antibody production after transfer to the irradiated recipients. Both cell type transfers gave rise to numerous pre-plasma cells within two to three days after transfer and to typical mature plasma cells a week afterwards. Apparently, various different kinds of cells in the lymphoid macrophage series are capable, on antigen stimulation, of producing pre-plasma cells and plasma cells which are capable of antibody production.

Dixon and his co-workers also provided evidence that it was the transferred lymphocytes which made the antibody.[61,62] Although serial morphologic studies seemed to show that the lymphocytes were the source of the developing pre-plasma and plasma cells, there had been no definite proof of antibody formation by the developing plasma cells in the transfer sites. However, Neil and Dixon[63] recently reported studies providing such evidence. They used the immunohistochemical technique of Coon to "visualize the appearance of antibodies to the specific antigen, and to correlate the

morphologic development of the subcutaneous cell-transfer sites following antigenic stimulation with the appearance and localization of antibody."

In their present studies, the authors observed specific antibody appearing within the pre-plasma cells and developing in the transfer sites. Connective tissue smears taken from cell transfer sites four hours after antigenic stimulation consisted chiefly of mature lymphocytes. During the rise of antibody synthesis a few days later, smears from the transfer sites showed a decrease in the number of lymphocytes and an increase in the number of "transitional pre-plasma, and plasma cells." Cells within which antibody at the injection sites was detected on the third day were morphologically similar to transitional and pre-plasma cells. The maximum amount of antibody-containing cells appeared on the fifth and sixth days. Following this, there was a marked preponderance of pre-plasma and plasma cells in the transfer sites. On the ninth day, the number of antibody-containing cells was greatly reduced. Also at this time, all of the remaining antibody-containing cells were mature plasma cells. This is in agreement with Fagraeus's hypothesis that the early forms of plasma cells predominate during the most active phase of antibody production, with the mature plasma cell appearing later.

Dixon and his associates believe that the host was reacting against the cells. Since their work shows that the transferred cells make the antibody, and since the antibody can be localized within developing plasma cells in the transfer site, they maintain that the plasma cells are derived from the transferred lymph node cells. "Considering the minimal mitotic activity in the transfer sites it would appear that during their antibody response the transferred lymphocytes metamorphose to the plasma cells without division via the stages described."

MECHANISM OF ANTIBODY INHIBITION

Cohn[64] injected fourteen-day-old chicken embryos with single doses of one of the following antigens: diphtheria toxoid, *E. coli* beta galactosidase, *E. coli* bacteriophage T2, pneumococci Type II, or arsanilic acid-beef serum albumen. None was successful in inhibiting the response of ten to twelve-week-old adult chickens to these antigens, *i.e.,* inhibiting antibody production.

Such a specific inhibition of antibody synthesis through embryonic contact is a symmetric analogue of specific induction, but not, according to Cohn, necessarily a consequence of either the template or modified enzyme theory. Under the one, it becomes a manifestation of continued presence of antigen; while under the other, it becomes a changed antibody-forming mechanism, unable to make certain configurations of antibody, with the alteration occurring most readily by contact with antigen during embryonic life. Cohn, on this point, states:

> As with the induction of antibody synthesis, the inhibition of antibody synthesis poses exactly the same problem as to the role of the antigen. In inhibition phenomena, the antigen might be considered an extra-cellular regenerated filter that prevents antibody from reaching the circulation, or an intra-cellular inhibitor. Of course, in the case of induction, by definition, the antigen is virtually acting intracellularly. By placing the inhibiting antigen in the cell, the distinction between the two hypotheses of inhibition becomes less clear, but we have not yet reached the point at which precise hypotheses to analyze this latter problem become necessary.

METABOLISM OF ANTIGEN-ANTIBODY COMPLEXES

Walter and Zipper[65] injected I^{131} beef gamma globulin-anti-beef gamma globulin complexes into the marginal vein of the ear of sensitized and normal animals. After studying the protein-bound radioactivity in the blood and

organs of these animals for seven days, they found, as has been found previously, that blood clearance takes place more rapidly in sensitized than in controlled animals. These authors agree with Dixon and Talmage that the elimination of antigen-antibody complex does not depend on tissue sensitization. "The mechanism of antigen-antibody complex removal seems essentially to be one either of filtration by the reticulo-endothelial organs, or of entrapment as emboli."

MULTI-SPECIFICITY OF NATURAL ANTIBODIES

The specificity of natural antibacterial antibodies has been studied by using the agglutination technique of red cells coated with bacterial polysaccharides.[66] The sera of all adult humans studied had antibodies against all strains under examination of the genera: Staphylococcus, Streptococcus, Sarcina, Mycobacteria, Corynebacterium, Escherichia, Klebsiella, Salmonella, Shigella, Serratia, and Bacillus. These antibodies appeared with the third month and continued to increase, although the umbilical cord blood did not contain antibacterial antibodies. At the same time as the titer of antibodies for other strains is reduced, antibodies for the homologous strain disappear in the absorption serum with red cells coated with bacterial polysaccharides. The eluates from coated and agglutinated red cells contained, in addition to homologous antibodies, antibodies for other bacteria. Saturating the sera with bacterial polysaccharides will suppress agglutination of red cells coated with homologous polysaccharides, but the agglutination titer of red cells coated with polysaccharides is ineffective for other microorganisms. Thus, this method can be used to differentiate strains of different genera and of different species within the same genus. Also, the saturation of eluates with bacterial polysaccharides suppresses only homologous agglutination.

Feliks Migrom and Zdzislawa Swiercynska[66] believe that their experimental findings are explained by Migrom's theory of the multispecificity of antibodies.

EFFECT OF ANTI-RABBIT LEUKOCYTE SERUM ON THE TRANSFER OF ANTIGEN-INCUBATED LYMPH NODE CELLS

It has been established that non-immune recipient rabbits develop antibodies to Shigella when they are injected with rabbit blood leukocytes that have been incubated with Shigella antigen. However, if recipient rabbits are pre-treated with leukocytes from donor rabbits and then are injected with antigen-incubated leukocytes, there is diminished antibody formation to Shigella. Because S. Harris[67] and his group believed that this was due to an immunologic reaction, they irradiated recipient rabbits in order to produce a decreased capacity for antibody formation. Then these rabbits were injected with leukocytes from a donor rabbit, while antigen-treated leukocytes from the donor were injected into the recipient. The Shigella antibody showed a higher titer than in non-irradiated controls. Irradiation had an inhibitory effect on pre-injected leukocytes.

Further tests were made with mature rabbits injected in the hind foot pads with donor rabbit leukocytes. After four days, the popliteal lymph nodes of the recipients were removed and made into a suspension. Then both antigen-incubated leukocytes from donor rabbits and cells from popliteal lymph nodes of recipient rabbits were injected into a second group of irradiated recipients. As the controls, these recipients had slower Shigella titers.

Harris and his associates also immunized rabbits to rabbit leukocytes and

injected their sera into recipients of antigen-incubated leukocytes. Although there was a marked suppression of resulting antibody titers, incubated anti-leukocyte sera with rabbit lymph node cells lessened the ability to interfere with antibody formation in the recipient. As a result, the titers were increased, and it was found that this capability was in the globulin fraction of the serum. These experiments show that the suppression of antibodies in recipients of antigen-incubated leukocytes can be passively transferred by anti-leukocyte serum.

REFERENCES

1. Godlowski, Z. Z.: Enzymatic Concept of Anaphylaxis and Allergy. Edinburgh and London: E. & S. Livingstone, Ltd., 1953.
2. Schick, B.: Physiologic and pathologic allergy. Ann. Allergy, 14:233 (May-June) 1956.
3. Boyd, W. C.: Fundamentals of Immunology. New York: Interscience Publishers, 1947.
4. Carpenter, P. H.: Immunology and Serology. Philadelphia: Saunders, 1956.
5. Doddi, G.: Allergia ed immunita nella tuberculosi. Le modificazioni del quadro sieroproteico dopo l'intradermoinierzione di tubercolina. Lotta Contral Tuberc., Roma, 24:149, 1954. Abstracted in Zentrabl. Bakt., Mar., 1957.
6. Sevag, M. D.: A new theory of allergic phenomena. Ann. Allergy, 13:233 (May-June) 1956.
7. Rich, A. R.: The Pathogenesis of Tuberculosis. Springfield, Illinois: Charles C Thomas, 1951.

7a. Rich, A. R.: Studies on hypersensitivity. Canad. M.A.J., 78:163 (Feb. 1) 1958.

8. Raffel, S. I.: The components of the tubercle bacillus responsible for the delayed type of "Infectious Allergy." J. Infec. Dis., 82:267, 1948.
9. Haurowitz, F.: Theories of Antibody Formation in the Nature and Significance of Antibody Response. A. M. Pappenheimer, ed. New York: Columbia University Press, 1953.
10. Haurowitz, F. and Crampton, C. F.: The fate in rabbits of intravenously injected I^{131} Endovoalbumin. J. Immunol., 68:73, 1952.
11. Burnet, F. M.: Enzyme, antigen, and virus. A Study of Macromolecular Pattern in Action. London: Cambridge Univ. Press, 1956.
12. Cushing, J. E. and Campbell, D. H.: Principles of Immunology. Chapter XIV. New York, Toronto, London: McGraw-Hill Book Company, Inc., 1957.
13. Pauling, L.: A theory of the structure and process of the formation of antibodies. J. Am. Chem. Soc., 62:2643, 1940.
14. Burnet, F. M. and Fenner, F.: The Production of Antibodies. London: MacMillan & Company, Ltd., 1949.
15. Stark, D. K.: Studies on pneumococcal polysaccharide. II. Mechanism involved in immunological paralysis. J. Immunol., 74:130, 1955. Cited by Burnet.
16. Mayer, R. L.: The significance of cross-links in the formation of Hapten carrier complexes. Internat. Arch. Allergy, Basel, 8:115, 1956.
17. Mayer, R. L. and Jaconia, D.: The role of the carrier in the formation of complete antigens. J. Allergy, 28:191 (May) 1957.
18. Mayer, R. L. and Jaconia, D.: The significance of fibroid protein carriers for the development of allergic skin reactions of the delayed type. Internat. Arch. Allergy, 10:13, 1957.
19. Campbell, Dan H.: Editorial. Some speculations on the significance of formation and persistence of antigen fragments in tissues of immunized animals. Blood, 12:589 (July) 1957.
20. Boyden, Stephen V.: Approaches to the Problem of Detecting Antibodies. Chapter 7 in Mechanisms of Hypersensitivity. Henry Ford Hospital International Symposium. Boston-Toronto: Little, Brown and Company, 1959.
21. McMaster, P. D.: The sites of antibody production. *In* Nature and Significance of the Antibody Response. A. M. Pappenheimer, Ed. New York: Columbia Univ. Press, 1953.
22. Chase, M. W.: Immunological reactions mediated through cells. *In* The Nature and Significance of the Antibody Response. A. M. Pappenheimer, Ed. New York: Columbia Univ. Press, 1953.

23. Chase, M. W.: The cellular transfer of cutaneous hypersensitivity. J. Bact., 51:63, 1946.
24. Chase, M. W.: The cellular transfer of cutaneous hypersensitivity to tuberculin. Proc. Soc., Exper. Biol. & Med., 59:134, 1945.
25. Chase, M. W., Pameshek, W., Haberman, S., Samter, M. and Squier, T. L.: The role of the formed elements of the blood in allergy and hypersensitivity. J. Allergy, 26:219 (May) 1955.
26. Raffel, S.: Bacterial hypersensitivity. J. Allergy, 27:199 (May) 1956.
27. Cooper, M. L., Keller, H. M. and Walters, E. W.: Microscopic characteristics of colonies of *Shigella flexneri 2a* and *2b* and their relation to antigenic composition, mouse virulence and immunogenicity. J. Immunol., 78:160 (Mar.) 1957.
28. Good, R. A. and Varco, H. L.: A clinical and experimental study of agammaglobulinemia. Lancet, 75:245, 1955.
29. Taliaferro, W. H. and Taliaferro, L. G.: Further studies on the radiosensitive stages in hemolysin formation. J. Infect. Dis., 95:134, 1954.
30. Taliaferro, W. H., Taliaferro, L. G. and Janssen, E. F.: Localization of x-ray injury to initial phases of antibody response. J. Infect. Dis., 91:105, 1952.
31. Harris, T. N. and Harris, S.: The genesis of antibodies. Am. J. Med., 20:114 (Jan.) 1956.
32. Bjorneboe, N., Gormser, H. and Lindquist, F.: Further experimental studies in the role of plasma cells as antibody producers. J. Immunol., 55:121, 1947.
33. Falchetti and Merieux, J.: Titration on rabbits and on tissue cultures of neutralizing antibodies of serums and milks of cows immunized by vaccinia virus. Rev. Path. Gen., 58:399, 1958.
34. Coons, A. H., Leduc, E. H. and Connolly, J. M.: Studies on antibody production. I. A method for the histochemical demonstration of specific antibody and its application to a study of the hyperimmune rabbits. J. Exper. Med., 102:49, 1955.
35. Wesslen, T.: Studies on the role of lymphocytes in antibody production. Acta dermat.-venereol., 32:265, 1952.
36. Roberts, J. C., Jr. and Dixon, F. J.: The transfer of lymph node cells in the study of the immune response to foreign proteins. J. Exper. Med., 102:379, 1955.
37. Wilson, G. S. and Miles, A. A.: Topley and Wilson's Principles of Bacteriology and Immunity, 4th ed., Vol. 11, p. 1261. Baltimore: The Williams & Wilkins Co., 1955.
38. Fagraeus, A.: Antibody production in relation to the development of plasma cells. Acta med. scandinav., suppl., 204, 1948.
39. Favour, C. B.: Cell injury in allergic inflammation. Internat. Arch. of Allergy & Appl. Immunol., 10:193, 1957.
40. Favour, C. B.: *In vitro* studies on cell injury in the tuberculin-type reaction: Implications in homotransplantation. Ann. New York Acad. Sci., 64:842, 1957.
41. Taliaferro, W. H. and Taliaferro, L. G.: The role of the spleen in haemolysin production in rabbits receiving multiple antigen injections. J. Infect. Dis., 89:143, 1951.
42. Maurer, P. H., Dixon, F. J. and Talmadge, D. W.: A quantitative comparison of antibody produced by x-radiated and normal rabbits. Proc. Soc. Exp. Biol., 83:163, 1953.
43. Harris, S., Harris, T. N. and Farber, M. B.: Studies on the transfer of lymph node cells. I. Appearance of antibody in recipients of cells from donor rabbits injected with antigen. J. Immunol., 72:148, 1954.
44. Harris, T. N., Harris, S. and Farber, M. B.: Transfer of x-irradiated rabbits of lymph cells incubated *in vitro* with *Shigella paradysenteriae*. Proc. Soc. Exper. Biol., 86:549, 1954.
45. Carrere, L. and Quatrefages, H.: Du transfert local de l'allergie a la melitiae. Compt. Remp. Acad. d. Sc., 234:369, 1952.
46. Gell, P. G.: On the Nature of Some Tissue Hypersensitivity Reactions in Physiopathology of the Reticulo-Endothelial System. A Symposium Organized by the Council for International Organizations of Medical Sciences. Springfield, Illinois: Charles C Thomas, 1957.
47. Doan, C. A.: The Reticulo-endothelial Cells in Health and Disease, in Physiopathology of the Reticulo-Endothelial System. A Symposium Organized by the Council for International Organizations of Medical Sciences. Springfied, Illinois: Charles C Thomas, 1957.
48. Yoffey, Joseph Mendel and Courtice, Frederick Colin: Lymphatics, Lymph and Lymphoid Tissue. Cambridge, Massachusetts: Harvard Univ. Press, 1956.

49. Coons, A. H.: *In* Symposium on the Nature and Significance of the Antibody Response. New York: Columbia Univ. Press, 1953.
50. Dixon, F. J., Bukantz, S. C., Dammin, G. P. and Talmadge, D. W.: *In* Symposium on the Nature and Significance of the Antibody Response. New York: Columbia Univ. Press, 1953.
51. Hawrowitz, S.: *In* Symposium on the Nature and Significance of the Antibody Response. New York: Columbia Univ. Press, 1953.
52. Spiers, R. S.: On the origin of plasma cells. Fed. Proc., 18:149, 1959.
53. Keohane, K. W. and Metcalf, W. K.: Changes in lymphocytes during antibody production. Nature, 183:195, 1959.
54. Fishman, M.: Antibody formation in tissue culture. Nature, 183:1200, 1959.
55. Pappenheimer, A. M., Jr., Scharff, M. and Uhr, J. W.: Delayed hypersensitivity and its possible relation to antibody formation. Chapter 25 *in* Mechanisms of Hypersensitivity. Henry Ford Hospital International Symposium. Boston-Toronto: Little, Brown and Company, 1959.
56. Fugino, S.: Studies on antibody production by peritoneal exudate cells. Agglutinin production in the recipient following transfer of donor's peritoneal exudate cells. Ann. Paed. Jap., 4:578, 1958 (In English).
57. Fugino, S.: Studies on antibody production by peritoneal exudate cells. Bacterial agglutinin synthesis by peritoneal exudate cells cultured with antigen *in vitro*. Ann. Paed. Jap., 4:444, 1958. (In English)
58. Swineford, O., Jr., Hoene, R., Quelch, S. and Samsell, D.: Purification of antibodies. I. Dextranase purification of antibody precipitated from Type II antipneumococcal rabbit serum by dextran. J. Allergy, 30:433, 1959.
59. Swineford, O., Jr., Quelch, S. and Samsell, D.: Dominant and recessive precipitating and desensitizing haptens. Ann. Allergy, 18:276 (Mar.) 1960.
60. Dixon, F. J.: Antibody synthesis. Paper presented at the Third International Congress of Allergology, Paris, France, October 21, 1958.
61. Roberts, J. C., Jr., Dixon, F. J. and Weigle, W. O.: Antibody-producing lymph node cells and peritoneal exudate cells. A.M.A. Arch. Path., 64:324, 1957.
62. Dixon, F. J., Weigle, W. O. and Roberts, J. C., Jr.: Comparison of antibody responses associated with the transfer of rabbit lymph node peritoneal exudate and thymus cells. J. Immunol., 78:56, 1957.
63. Neil, A. L. and Dixon, F. J.: Immunohistochemical detection of antibody in cell-transfer studies. A.M.A. Arch. Path., 67:643, 1959.
64. Cohen, M.: The problem of specific antibody inhibition of antibody synthesis in adult animals by immunization of embryos. Ann. New York Acad. Sc., 64:859, 1957.
65. Walter, H. and Zipper, H.: The metabolism of some antigen-antibody complexes in the rabbit. J. Immunol., 82:12, 1959.
66. Migrom, Feliks and Swiercynska, Z.: Multispecificity of natural antibodies. Arch. Immunol. Terapii Doswiadczalnej. Tom., 3:367, 1955. Abstracted in Rev. Allergy, 13:55, 1959.
67. Harris, S., Harris, T. N. and Farber, M. B.: Studies on the transfer of lymph node cells. XII. The effect of anti-rabbit leukocyte serum on the transfer of antigen-incubated lymph node cells. J. Exper. Med., 108:411, 1958.

(To be continued)

Papers of Interest

Lennette, E. H., Flintjer, J. D., Culver, J. O., Fox, V. L. and Stevens, T. E.: Adenovirus vaccine: evaluation of antibody response and protective efficacy and comparison with an earlier preparation. Am. J. Hyg., 71:193 (Mar.) 1960.

Response to types 4 and 7 adenoviruses was good as measured by neutralizing antibody responses. That to type 3 was poor.

Schultz, I., Friedman, M. and Pierce, W. E.: Relationship of adenoviruses to respiratory illness in ten naval recruits sampled repeatedly during training.

In ten naval recruits, serum was checked frequently during their training period. It was shown that either other agents or factors must be responsible for the chronic respiratory disorders often experienced.

Paddison, R. M., Anthony, S., Ferriss, G. S. and Welsh, R. A.: Role of antihistamine therapy in diphenyl hydantoin-induced gingival hyperplasia. Clin. Pharmacol. and Therap., 1:316 (May-June) 1960.

In a study of 35 patients, no visual or histopathological significant effect was noted following the use of chlorprophenpyridamine maleate upon the gingival hyperplasia induced by diphenylhydantoin administration.

Somner, A. R., Rogan, M. C. and Grant, I. W. B.: Prolonged corticosteroid therapy in chronic asthma. Brit. M. J., 1:1092 (Apr. 9) 1960.

Six of fourteen patients treated with prednisolone or triamcinolone for a period of fifteen months to three years showed an unsatisfactory response to treatment. Sustained improvement was noted in the eight others.

Goldblatt, S.: Severe reaction to griseofulvin. J.A.M.A., 172:1643 (Apr. 9) 1960.

Urticaria and joint pains in a thirty-year-old male patient occurred following treatment with griseofulvin for T. rubrum infection of the skin. Response was noted in three weeks to treatment with antihistaminic agents.

Kahlson, G., and Rosengren, E.: Inhibition of histamine formation and some of its consequences. J. Physiol, 149 (Dec.) 1959.

Where, in pregnant rats, a pyridoxine deficient, histamine-free diet and formation of histamine is prevented by injections of semicarbazide, the foetuses all die.

Mota, I. and da Silva, W. Dias: Antigen-induced damage to isolated sensitized mast cells. Nature, 186:245 (Apr. 16) 1960.

Isolated and washed mast cells taken from sensitized animals can respond to exposure to specific antigens. It is suggested that when in anaphylactic shock such cells are damaged, then the antigen-antibody reaction must occur within the mast cell.

Bhoola, K. D. and Schachter, M.: A comparison of serum kallikrein, bradykinin and histamine on capillary permeability in the guinea pig. J. Physiol 149 (Dec.) 1959.

It appears that kallidin which resembles bradykinin medicates the action of kallikrein which when in the serum increases the permeability of capillaries.

Jaroslow, B. N.: Factors associated with initiation of the immune response. J. Infectious Dis., 107:56-64 (July-Aug.) 1960.

Antibody synthesis is hypothesized as occurring during a specific stage of mitosis.

Nevins, D. M.: Anaphylactoid reaction following administration of one meprobamate tablet. Ann. Internal Med., 53:192-193 (July) 1960.

An anaphylactoid reaction followed the ingestion of meprobamate 400 mg Hydrocortisone administered intravenously was followed by dramatic relief.

Campbell, D. H. and Garvey, J. S.: Factors involved in antibody formation. J. Infectious Dis., 107:15-28 (July-Aug.) 1960.

Old Master gives emphasis to the fact of how much there is yet to learn.

IVth International Congress of Allergology

Hotel Commodore, New York City, October 15-20, 1961

Scientific Program

SUNDAY, October 15, 1961

P.M.
2:00 CONVOCATION
Welcoming addresses
By Prominent Government Officials
RESEARCH IN ALLERGOLOGY:
Dr. Justin M. Andrews
National Institute of Allergy and Infectious Diseases
Introductory Remarks by Dr. Francis B. Rackemann, Honorary President, IVth International Congress of Allergology
ROBERT A. COOKE, PIONEER IN ALLERGY:
Professor Pasteur Vallery-Radot
ORGANIZATION OF THE CONGRESS:
Dr. William B. Sherman
THE ACHIEVEMENTS AND THE PERSPECTIVES OF OUR CONGRESSES
Dr. Bernard N. Halpern, President, International Association of Allergology; President, IVth International Congress of Allergology

5:30 to 7:00 RECEPTION at Hotel Commodore for members, wives and guests.

MONDAY, October 16, 1961

A.M. *Chairman:* Professor Pasteur Vallery-Radot (France)
CONFERENCE: UNITY AND HETEROGENICITY OF ANTIBODIES: Dr. M. Heidelberger (U. S. A.)

SYMPOSIUM: ANTIGEN-ANTIBODY REACTIONS IN HYPERSENSITIVITY
Antibody Structure: Dr. R. R. Porter (Gt. Britain)
Sensitization Process: Dr. B. N. Halpern (France)
Biochemical Mediators: Dr. J. H. Humphrey (Gt. Britain)
Histological Sequences of the Antigen-Antibody Reaction: Dr. E. Letterer (Germany)

P.M. *Chairman:* Dr. Bernard N. Halpern (France)
CONFERENCE: ANTIGEN STRUCTURE: Dr. E. Kabat (U. S. A.)
PANEL: DRUG HYPERSENSITIVITY
Chemical Considerations: Dr. M. W. Chase (U. S. A.)
Immune Mechanisms in Drug Hypersensitivity: Dr. M. Samter (U. S. A.)
Immunological Studies on Serum Sickness: Dr. C. E. Arbesman (U. S. A.)
Clinics and Pathology in Drug Hypersensitivity: Dr. C. Jiménez Díaz (Spain)
Penicillin Hypersensitivity: Dr. B. B. Siegel (U. S. A.)
Prophylaxis and Treatment in Drug Hypersensitivity: Dr. U. Serafini (Italy)

TUESDAY, October 17, 1961

A.M. *Chairman:* Dr. Bram Rose (Canada)
CONFERENCE: THE IMMUNE RESPONSE IN HYPERSENSITIVITY: Dr. S. Raffel (U. S. A.)

PANEL: DESENSITIZATION
Status of Knowledge: Dr. W. B. Sherman (U. S. A.)
Treatment with Injections of Aqueous Allergenic Extracts: Dr. F. Lowell (U. S. A.)

Treatment with Emulsified Allergenic Extracts (Repository Treatment): Dr. M. Loveless, Dr. S. M. Feinberg, Dr. E. A. Brown, Dr. C. E. Arbesman (U. S. A.)

P.M. SECTIONAL MEETINGS
(Titles and Authors for all Sectional Meetings to appear in Final Program)

8:00 RECEPTION at the Metropolitan Museum of Art for members and wives.

WEDNESDAY, October 18, 1961

A.M. *Chairman:* Dr. C. Jiménez Díaz (Spain)

CONFERENCE: HYPERSENSITIVITY AS A PROBLEM OF GENERAL BIOLOGY: Sir Macfarlane Burnet (Australia)

PANEL: DELAYED HYPERSENSITIVITY

Delayed Hypersensitivity to Bacterial and Tissue Antigens: Dr. H. W. Lawrence (U. S. A.)
Delayed Hypersensitivity to Soluble Proteins: Dr. S. B. Salvin (U. S. A.)
Delayed Hypersensitivity to Simple Chemicals: Dr. H. N. Eisen (U. S. A.)
Delayed Hypersensitivity to Tuberculosis Bacilli: Dr. R. Kourilsky (France)
Delayed Hypersensitivity to Fungal Antigens: Dr. W. Jadassohn (Switzerland)

P.M. SECTIONAL MEETINGS

THURSDAY, October 19, 1961

A.M. *Chairman:* Dr. E. Letterer (Germany)

CONFERENCE: IMMUNOLOGICAL MECHANISMS IN HOMOGRAFT REJECTIONS: Dr. R. E. Billingham (Great Britain)

PANEL: AUTOSENSITIZATION AND ISOSENSITIZATION

Hypogammaglobulinemia and Immunological Responses: Dr. C. A. Janeway (U. S. A.)
Isosensitization to Nervous Substances: Dr. B. H. Waksman (U. S. A.)
Autosensitization to Thyroid: Dr. E. Witebsky (U. S. A.)
Autosensitization to Vascular Antigens: Dr. D. Pressman (U. S. A.)
Studies on Inhibition of Immune Response: Dr. J. Sterzl (Czechoslovakia)

P.M. SECTIONAL MEETINGS

7:00 BANQUET at the Hotel Commodore for members and wives.

FRIDAY October 20, 1961

A.M.

CONFERENCE: CRITICAL REVIEW OF METHODS USED IN IMMUNOLOGY AND ALLERGY: Dr. D. Talmage (U. S. A.)

PANEL: ADVANCES IN METHODS OF EXPERIMENTAL HYPERSENSITIVITY

Hemagglutination Technics: Dr. A. Sehon (Canada)
Gel Diffusion: Dr. J. Oudin (France)
Immunoelectrophoresis: Dr. J. Burtin (France)
Fluorescent Labelling: Dr. M. H. Kaplan (U. S. A.)
Passive Cutaneous Anaphylaxis: Dr. Z. Ovary (U. S. A.)
Complement Fixation: Dr. O. Bier (Brazil)

P.M.

CONFERENCE: USES AND ABUSES OF STEROIDS IN ALLERGIC DISEASES: Dr. R. S. B. Pearson (Great Britain)

SYMPOSIUM: THERAPEUTICS

Management of Intractable Asthma: Dr. A. Frankland (Great Britain)
Institutional Treatment of Asthma: Dr. S. C. Bukantz (U. S. A.)
Management of Atopic Dermatitis: Dr. R. L. Baer (U. S. A.)
Psychotherapy in Allergy: Dr. H. H. Abramson (U. S. A.)
Psychosomatic Group Therapy with Parents of Children with Intractable Asthma: Dr. M. M. Peshkin (U. S. A.)

Topics and Authors of Abstracts for the Sectional Meetings

I. THE ROLE OF PHARMACOLOGICALLY ACTIVE SUBSTANCES

E. Middleton, Jr., et al (U. S. A.); S. E. Lindell, et al (Sweden); D. J. Gocke, et al (U. S. A.); P. Stern, et al (Yugoslavia); S. Vukobratovic (Yugoslavia); K. Jensen (Denmark); R. Panzani (France); J. L. Parrot, et al (France); R. Benda (France); P. Wodniansky (Austria); I. Joo, et al (Hungary); H. S. Novey (U. S. A.).

II. HISTAMINE AND SEROTONIN ANTAGONISTS

J. R. Castaño (Portugal); W. Gronemeyer, et al (Germany); B. von Boros, et al (Germany); H. H. Gelfand, et al (U. S. A.); P. E. Siegler, et al (U. S. A.); P. Naranjo (Ecuador); A. Kaminsky, et al (Argentina); S. Karady (Hungary).

III. WANDERING CELLS IN ALLERGY

R. K. Archer, et al (Great Britain); M. M. El-Mehairy, et al (Egypt); H. Herxheimer (Germany); H. Rorsman (Sweden); G. Salvato (Italy).

IV. PHYSIOLOGICAL PATTERNS IN ALLERGIC DISORDERS

F. W. Wittich, et al (U. S. A.); F. Sicuteri, et al (Italy); T. G. Randolph (U. S. A.); P. Jean, et al (Canada); H. Colldahl, et al (Sweden); G. F. Harsh, et al (U. S. A.); R. Laborie, et al (France); L. Michelet, et al (France); E. A. Brown (U. S. A.); M. A. Green (U. S. A.); M. D. Sanger (U. S. A.); C. Huriez, et al (France).

V. ASTHMA

F. H. Milner, et al (Great Britain); A. Bouhuys (The Netherlands); A. H. Rowe, et al (U. S. A.); M. Mimica (Yugoslavia); W. Leith, et al (Canada); E. I. Cohen (Romania); O. Loras (France); H. Marynowska-Kaulbersz, et al (Poland); A. W. Frankland (Great Britain); E. Mendes (Brazil); J. D. Favre (Switzerland); D. L. Blumenthal, et al (U. S. A.); O. Swineford, Jr., et al (U. S. A.); R. J. Moriarty, et al (U. S. A.); S. Siegal, et al (U. S. A.); M. M. Miller, et al (U. S. A.).

VI. ALLERGIC MANIFESTATIONS

L. Unger, et al (U. S. A.); L. L. Kulczycki, et al (U. S. A.); A. Tomatis (France); J. Harkavy, et al (U. S. A.); S. Siegal (U. S. A.); A. P. Friedman, et al (U. S. A.); P. Naranjo, et al (Ecuador); K. Linser (Germany); H. G. Rapaport (U. S. A.); A. H. Rowe, et al (U. S. A.); S. Zvi Kantor (Israel); C. Huriez, et al (France); S. J. Appel, et al (U. S. A.); I. Engström (Sweden); L. S. Girsh (U. S. A.); B. A. Berman (U. S. A.); B. Redner, et al (U. S. A.); J. H. Fries, et al (U. S. A.); G. Filipp, et al (Germany); B. F. Feingold, et al (U. S. A.); G. G. Springer, et al (U. S. A.); M. W. Chase, et al (U. S. A.); M. Soriano (Spain); V. L. Szanton, et al (U. S. A.).

VII. STEROID THERAPY

C. J. Falliers, et al (U. S. A.); E. L. Keeney, et al (U. S. A.); B. Rose (Canada); M. M. El-Mehairy, et al (Egypt); R. G. Evans (Great Britain); M. K. Hajos (Hungary); G. Gerchunof, et al (Argentina); B. Lindholm, et al (Sweden); C. M. Kohn (U. S. A.); S. Friedlaender, et al (U. S. A.); K. Linser (Germany); B. Lindholm (Sweden); J. R. Vaccarezza (Argentina).

VIII. AUTOIMMUNE DISEASES

I. L. Bernstein, et al (U. S. A.); E. F. Pfeiffer (Germany); K. Rother, et al (Germany); D. E. Johnstone (U. S. A.); E. Helander (Sweden); A. L. Sherwin, et al (Canada); B. Steiner (Hungary).

IX. IMMUNOLOGY OF CANCER

G. A. Koelsche (U. S. A.); C. Tal, et al (Israel); L. Adelsberger, et al (U. S. A.); E. W. Fisherman (U. S. A.); J. G. Makari (U. S. A.).

X. MICROBIAL AGENTS AS ALLERGENS

A. Liebeskind (Israel); J. G. Feinberg, et al (Great Britain); D. Merksamer, et al (U. S. A.); H. M. Brown, et al (Great Britain); K. M. Citron (Great Britain); J. Pepys (Great Britain); C. Benaim-Pinto (Venezuela); G. Centanni, et al (Italy); R. Barkai-Golan (Israel); S. F. Hampton, et al (U. S. A.); M. Dworetzky, et al (U. S. A.); S. W. Simon, et al (U. S. A.); G. H. Waddell, et al (U. S. A.); K. A. Baird (Canada); H. Blatt (U. S. A.).

XI. CONSTITUENTS OF RAGWEED POLLEN

K. J. Lea, et al (Canada); M. Gershenfeld, et al (U. S. A.).

XII. HYPOSENSITIZATION TO POLLENS

H. S. Baldwin, et al (U. S. A.); G. E. Gaillard (U. S. A.); G. Sobel (U. S. A.); S. J. Prigal (U. S. A.); W. H. Lipman, et al (U. S. A.); E. R. Pons, Jr. (U. S. A.); M. Sanchez-Medina (Colombia); J. Tabart, et al (France); M. M. Gould (U. S. A.); M. Franciulli (Argentina).

XIII. ANTIBODIES IN ATOPIC AND REAGINIC ALLERGIES

D. Lidd, et al (U. S. A.); R. J. Feinberg, et al (U. S. A.); L. Perelmutter, et al (Canada); A. E. O. Menzel, et al (U. S. A.); E. P. Crump, et al (U. S. A.); J. T. Connell, et al (U. S. A.); L. Gyenes, et al (Canada); H. Blumer, et al (Canada); A. H. Rosenblum, et al (U. S. A.); N. Lass (Israel); H. S. Bernton, et al (U. S. A.).

XIV. HYPERSENSITIVITY TO CHEMICAL ALLERGENS (CONTACT DERMATITIS, DRUG ALLERGY)

T. Miyazawa (Japan); A. Oehling, et al (Germany); G. L. Waldbott (U. S. A.); F. J. Farrerons-Co, et al (Spain); M. Grolnick (U. S. A.); K. H. Schulz (Germany); C. Baena Cagnani (Argentina); Y. Oshima, et al (Japan); S. O. Freedman, et al (Canada); T. Torii, et al (Japan); R. H. Schwartz, et al (U. S. A.); H. T. Friedman (U. S. A.); L. Maslansky (U. S. A.); J. A. Moretti (Uruguay).

XV. BASIC IMMUNOLOGY

K. L. Burdon (U. S. A.); O. L. Frick, et al (France); N. Chakravarty (Venezuela); R. A. Binaghi, et al (France); H. V. Huidobro (Argentina); P. Liacopoulos, et al (France); G. F. Mikulicich, et al (U. S. A.); E. Rodriguez, et al (U. S. A.); W. Boke, et al (Germany); A. G. Osler, et al (U. S. A.); G. Goldstein, et al (U. S. A.); E. H. Relyveld, et al (France); O. Swineford, Jr., et al (U. S. A.); V. S. Uzelatz (Yugoslavia); U. Rother, et al (Germany); G. E. Davies, et al (Great Britain); H. Friedman (U. S. A.); K. Ishizaka, et al (Japan); J. J. Kraut (U. S. A.); J. Prochazka Fisher (U. S. A.); A. B. Vivera, et al (Philippines and U. S. A.); Mme. F. Laborie, et al (France); B. Schick, et al (U. S. A.); J. A. Flick (U. S. A.); S. B. Salvin, et al (U. S. A.); J. Johanovsky, et al (Czechoslovakia); M. Yamanaka (Japan); T. G. Kovats, et al (Hungary); F. Celada, et al (U. S. A.); O. Vejbora, et al (Czechoslovakia).

News Items

AMERICAN COLLEGE OF ALLERGISTS
EIGHTEENTH ANNUAL CONGRESS

Hotel Radisson

Minneapolis, Minnesota

April 4, 5, 6, 1962

Fellows wishing to appear on the program should submit abstracts in quadruplicate, limited to 250 to 300 words, and accompanied by a 35 to 40 word resumé-summary to Dr. Mayer A. Green, 6112 Jenkins Arcade, Pittsburgh 22, Pa., prior to November 15, 1961.

Associate Fellows are urged to submit papers in competition for the BELA SCHICK AWARD granted through the Women's Auxiliary. Instructions are the same as above.

The CLEMENS VON PIRQUET AWARD, comprising a prize of $250 and a Certificate of Award, will be presented to the Intern, Resident or Medical Student submitting the best paper on any aspect of allergy or related fields of medicine. Submit entire manuscript in quadruplicate to Dr. Green before November 15, 1961. The winning essayist need not be present to receive the Award.

Fellows, Associate Fellows and non-members wishing to display SCIENTIFIC EXHIBITS April 3 to 5, are requested to send brief summaries and descriptions in duplicate to Dr. Green before December 15, 1961.

PLEASE SUBMIT PAPERS AS SOON AS POSSIBLE.

AMERICAN COLLEGE OF ALLERGISTS
GRADUATE INSTRUCTIONAL CONGRESS

Hotel Radisson

Minneapolis, Minnesota

April 1, 2, 3, 1962

Several SCHOLARSHIPS are being generously underwritten by the Women's Auxiliary.

Applications from physicians for these SCHOLARSHIPS should be sent to Dr. Mayer A. Green, 6112 Jenkins Arcade, Pittsburgh 22, Pa. Applications from interns and residents must be accompanied by a letter of approval from the Medical Director or comparable official from the hospital they are serving.

PROMOTION TO ACTIVE FELLOWSHIP

All applications for promotion to Active Fellowship in the American College of Allergists must be completed and returned to John D. Gillaspie, M.D., Treasurer, 2141 Fourteenth Street, Boulder, Colorado, on or before *December 31, 1961,* in order to be considered for promotion at the Annual Meeting in Minneapolis, Minnesota, April 1 to 6, 1962.

The examination for promotion will be given on Tuesday, April 3, 1962.

NEWS ITEMS

AMERICAN FORUM ON ALLERGY

The American Forum on Allergy announces that a meeting of this organization will be held at the Star Dust Hotel, Las Vegas, Nevada, on Monday, Tuesday and Wednesday, December 4, 5, and 6, 1961.

All those wishing information should send their requests to Dr. Leon Unger, 185 North Wabash Avenue, Chicago 1, Illinois.

CALIFORNIA SOCIETY OF ALLERGY

The joint annual meeting of the Allergy Section of the California Medical Association and the California Society of Allergy was held on April 30, 1961, in Los Angeles, California.

The following officers were elected for 1961-1962:

President.....Garner S. Stout, M.D.
Secretary-Treasurer.....Jerome J. Sievers, M.D.
Assistant Secretary.....Walter R. MacLaren, M.D.

CHICAGO SOCIETY OF ALLERGY

At the last meeting of the Chicago Society of Allergy the following officers were elected:

President.....Charles M. Jenkins, M.D.
President-Elect.....Abe Matheson, M.D.
Secretary-Treasurer.....Ethel M. Davis, M.D.

ON CHROMOSOMES

For example, with only twenty-three chromosomes in the human paternal and maternal germ cells, the total number of different combinations of chromosomes in the potential offspring of one man and one woman is over 70 thousand billions, or 26,000 times the population of the earth. But every chromosome is composed of many genes, each of which may have several variations: on the assumption that there are a thousand distinctive genes in the human chromosomes (and there are probably many more), and that each gene has only two variations (and there are probably more), it has been calculated that the number of potential combinations of genes to be derived from one man and one woman is 2 to the 1000th power, a figure greater than the number of electrons in the world. With random mating such as occurs in nature, it can therefore be safely said that no two individuals in any species are ever exactly alike. This even applies to identical twins, which *may,* but do not necessarily, start out with an absolutely identical genetic pattern but are now thought to undergo some genetic differentiation during embryonic development.—From *From Fish to Philosopher* by HOMER W. SMITH. Ciba edition, revised (Summit, N. J.) with permission of Little, Brown, & Co., Boston, Massachusetts, 1959.